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NOTE

Is there a cold shock response in the Antarctic psychrophile *Pseudoalteromonas haloplanktis*?

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Abstract The growth behavior and the proteomic response after a cold shock were investigated in the psychrophilic Antarctic bacterium *Pseudoalteromonas haloplanktis*. Remarkably, no cold-induced proteins were observed in the proteome, whereas some key proteins were repressed. This suggests noticeable differences in the cold shock response between a true psychrophile and mesophiles.

Keywords Psychrophiles · Antarctic · Cold shock · Proteomics

In microorganisms, a cold shock response is induced by transferring a culture run at optimal temperature to a lower but still permissive temperature. The subsequent cellular response to these temperature downshifts have been extensively described for *E. coli* and *B. subtilis* (Graumann and Marahiel 1996; Phadtare et al. 1999; Ermolenko and Makhatadze 2002; Weber and Marahiel 2003; Phadtare 2004). In *E. coli* for instance, both growth and protein synthesis are arrested after a cold shock from 37 to 15 °C, then a set of cold-induced proteins is transiently synthesized before growth resume after 2–4 h (Supplementary Fig. S1). The function of these cold-induced proteins has been related to the microorganism adaptation to the new cold-culture conditions. The cold shock response was also analyzed in psychrotrophs, i.e. microorganisms growing optimally at near-mesophilic temperatures but tolerating cold temperatures for division. Temperature downshifts from 30–35 to 5–10 °C in psychrotrophs revealed a response qualitatively similar to that of mesophiles, with the noticeable exception of the continuous overexpression of cold acclimation proteins during sustained growth in the cold (Hebraud and Potier 1999; Inouye and Phadtare 2007; Phadtare and Inouye 2008). These cold acclimation proteins were expected to be key determinants for cold tolerance.

The cellular adaptations to low temperatures in true psychrophiles (i.e. permanently and successfully thriving in constantly cold environments) have been recently investigated by various modern proteomic methods (for references, see Piette et al. 2011b). For instance, the Antarctic bacterium *P. haloplanktis* grown at low temperature overexpresses enzymes involved in protein synthesis and folding and regulates its cytoplasmic redox balance as a result of improved oxygen solubility. Furthermore, the psychrophilic bacterium strongly represses the synthesis of heat shock chaperones, depresses its general metabolism and avoids pathways involving metal ions that are prone to reactive oxygen species production (Piette et al. 2010, 2011a). However, for all psychrophiles studied so far, proteomes were compared using cells growing sustainably at a low and a high temperature. Accordingly, and to the best of our knowledge, an authentic cold shock response has not been reported for true psychrophiles and this prompted us to investigate this aspect for the Antarctic

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psychrophile *P. haloplanktis* TAC 125. It should be stressed that inducing a cold shock in a psychrophilic bacterium is challenging. Indeed, in a model microorganism such as *E. coli* (Inouye and Phadtare 2004), a moderate cold shock (from 37 to 25 °C) results in instantaneous shift towards the new growth and metabolic regime. On the other hand, severe downshifts at non-permissive temperatures (4 °C) result in growth arrest, without further resumption. By contrast, temperature downshifts at low but permissive temperatures (10–15 °C) induce a cold shock characterized by a growth arrest and an acclimation phase during which cold shock proteins are overexpressed in order to promote growth resumption (Supplementary Figure S1). Accordingly, our goal was to find conditions inducing an acclimation phase and to analyze the proteomic adjustments during this phase.

Pseudoalteromonas haloplanktis displays doubling times of 1 h 40 min at 18 °C, of 4 h at 4 °C and of 5 h 15 min at 0 °C (Piette et al. 2011a). When cultures were shifted from 18 to 4 °C, the bacterium immediately reduced its growth rate to a value similar to that recorded for sustained growth at 4 °C. The lack of growth arrest, or lag phase typical of a cold shock response, indicates that such temperature downshift is not very stressful for the Antarctic strain. By contrast, when cultures were transferred from 18 to 0 °C (in melting ice with further incubation and shaking at 0 °C) a lag phase of about 6–7 h was observed before slow resumption of growth (Fig. 1). This indicates that growth arrest of this strain can only be induced by a severe temperature downshift reaching near-freezing temperatures.

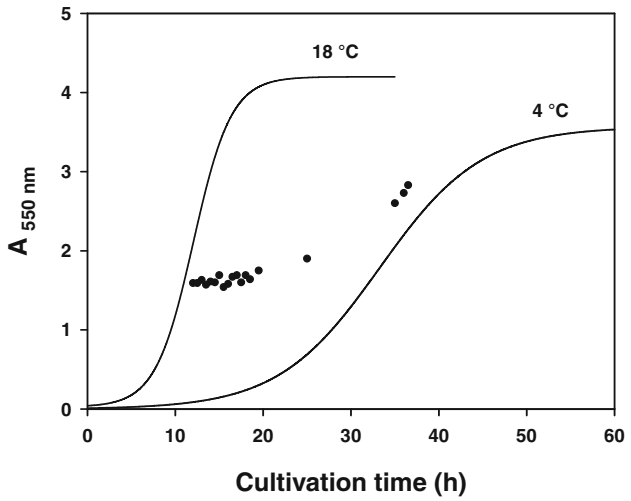

Fig. 1 Growth behavior of *P. haloplanktis* TAC 125. *Solid lines* represent typical growth curves at 4 and 18 °C (Piette et al. 2011a). A cold shock to 0 °C has been performed after 10 h at 18 °C. *Closed symbols* illustrate a representative culture evolution at 0 °C

The proteomes expressed by the Antarctic bacterium before and after the cold shock from 18 to 0 °C were compared by two-dimensional differential in-gel electrophoresis (2D-DIGE) as described previously (Piette et al. 2010). This method enables the co-migration in equal amounts of cell extracts obtained from both conditions (labeled by distinct CyDye fluorophores) in triplicate gels. Spots with low molecular mass were also checked with Tricine–Tris gels (Schagger 2006). Cell extracts prepared before the shock and 1 h after the cold shock failed to reveal any statistical variation amongst the ~3,000 spots typically detected in a 2D-DIGE experiment, indicating a simple growth arrest without significant cellular adjustments at the proteome level. Unexpectedly, when the comparison was made using cell extracts prepared 6 h after the cold shock, no cold-induced proteins were detected (i.e. proteins that are more abundant after the shock). By contrast, 17 protein spots were found to be repressed after the shock (with repression factor higher than 1.5×), including 5 spots with repression factors higher than 2×. This demonstrates a moderate and discrete response of the proteome in the Antarctic strain.

Amongst these cold shock-repressed spots, 5 contained proteins which satisfied statistical biological variation analysis and yielded significant mass spectrometry (peptide mass fingerprint and MS/MS) identification scores. These proteins are listed in Table 1 (technical and statistical data are provided in the Supplementary Table S1). Interestingly, all the identified cold shock-repressed proteins have been previously identified as cold-repressed proteins during sustained growth of *P. haloplanktis* at 4 °C (Table 1). This strongly suggests that the main cold shock response corresponds to a slow shift towards cellular adjustments already described for cells growing at 4 °C and involving, for instance, the repression of heat shock proteins (as exemplified by GroEL in Table 1) and reduction of the metabolism and biomass (4 other proteins).

Table 1 Cold shock-repressed proteins identified in *P. haloplanktis* TAC 125

Protein	Down-regulation ratio	
	After cold shock	During sustained growth at 4 °C[a]
GroEL protein	1.55	3.41
4-Hydroxyphenylpyruvate dioxygenase	1.59	1.83
F0F1 ATP synthase subunit alpha	1.88	1.72
Peroxiredoxin 2	3.48	15.66
Elongation factor Tu	3.91	13.14

[a] Data from Piette et al. (2011a)

The lack of a typical cold shock response under our temperature downshift conditions is puzzling. One cannot exclude the possibility that the psychrophilic Antarctic bacterium constitutively synthesizes cellular proteins aimed at relieving the detrimental effects of possible temperature downshifts. Furthermore, such bacteria are expected to become entrapped in the winter ice pack at −20 °C (Deming 2002). Accordingly, a cold shock from 4 to −20 °C could be more environmentally relevant, but is also technically challenging. It is expected that our observations will stimulate similar experiments using other psychrophiles that are currently under proteomic investigations.

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References

Deming JW (2002) Psychrophiles and polar regions. Curr Opin Microbiol 5:301–309

Ermolenko DN, Makhatadze GI (2002) Bacterial cold-shock proteins. Cell Mol Life Sci 59:1902–1913

Graumann P, Marahiel MA (1996) Some like it cold: response of microorganisms to cold shock. Arch Microbiol 166:293–300

Hebraud M, Potier P (1999) Cold shock response and low temperature adaptation in psychrotrophic bacteria. J Mol Microbiol Biotechnol 1:211–219

Inouye M, Phadtare S (2004) Cold shock response and adaptation at near-freezing temperature in microorganisms. Sci STKE 2004:pe26

Inouye M, Phadtare S (2007) The cold-shock response. In: Gerday C, Glansdorff N (eds) Physiology and biochemistry of extremophiles. ASM Press, Washington, DC, pp 180–193

Phadtare S (2004) Recent developments in bacterial cold-shock response. Curr Issues Mol Biol 6:125–136

Phadtare S, Inouye M (2008) Cold-shock proteins. In: Margesin R, Schinner F, Marx JC, Gerday C (eds) Psychrophiles, from biodiversity to biotechnology. Springer, Berlin, pp 191–209

Phadtare S, Alsina J, Inouye M (1999) Cold-shock response and cold-shock proteins. Curr Opin Microbiol 2:175–180

Piette F, D'Amico S, Struvay C, Mazzucchelli G, Renaut J, Tutino ML, Danchin A, Leprince P, Feller G (2010) Proteomics of life at low temperatures: trigger factor is the primary chaperone in the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125. Mol Microbiol 76:120–132

Piette F, D'Amico S, Mazzucchelli G, Danchin A, Leprince P, Feller G (2011a) Life in the cold: a proteomic study of cold-repressed proteins in the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125. Appl Environ Microbiol 77:3881–3883

Piette F, Struvay C, Feller G (2011b) The protein folding challenge in psychrophiles: facts and current issues. Environ Microbiol 13:1924–1933

Schagger H (2006) Tricine-SDS-PAGE. Nat Protoc 1:16–22

Weber MH, Marahiel MA (2003) Bacterial cold shock responses. Sci Prog 86:9–75