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ORIGINAL PAPER

Proteomic studies of an Antarctic cold-adapted bacterium, *Shewanella livingstonensis* Ac10, for global identification of cold-inducible proteins

Jun Kawamoto · Tatsuo Kurihara · Masanari Kitagawa · Ikunoshin Kato · Nobuyoshi Esaki

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Abstract Proteomic analysis of a cold-adapted bacterium, *Shewanella livingstonensis* Ac10, isolated from Antarctic seawater was carried out to elucidate its cold-adaptation mechanism. The cells were grown at 4°C and 18°C, and soluble and membrane proteins were analyzed by two-dimensional gel electrophoresis. At 4°C, the relative abundance of 47 soluble proteins and five membrane proteins increased more than twofold, and these proteins were analyzed by peptide mass fingerprinting. Twenty-six soluble proteins and two membrane proteins were identified. These included proteins involved in RNA synthesis and folding (RpoA, GreA, and CspA), protein synthesis and folding (TufB, Efp, LysU, and Tig), membrane transport (OmpA and OmpC), and motility (FlgE and FlgL). Cold-inducible RpoA, GreA, and CspA may be required for efficient and accurate transcription and proper folding of RNA at low temperatures, where base pairing of nucleic acids is stable and undesired secondary structures of RNA tend to form. Tig is supposed to have peptidyl-prolyl *cis–trans* isomerase activity and facilitate proper folding of proteins at low temperatures. The cold induction of OmpA and OmpC is likely to counteract the low diffusion rate of solutes at low temperatures and enables the efficient uptake of nutrients. These results provided many clues to understand microbial cold-adaptation mechanisms.

Keywords Cold-adapted bacterium · Cold adaptation · Cold-inducible proteins · Proteomics · *Shewanella livingstonensis*

Abbreviations

PMF	Peptide mass fingerprinting
2DE	Two-dimensional gel electrophoresis

Introduction

The biosphere of the earth is dominated by permanently cold environments such as the Polar Regions, deep seas and high mountains. Many microorganisms inhabit these environments by adapting to low temperatures. Such cold-adapted microorganisms have been attracting a great deal of attention from both fundamental and application points of view (Cavicchioli et al. 2002; D'Amico et al. 2006). Cold-active enzymes from these microorganisms are expected to be useful as food-processing enzymes, additives to detergents, and tools for molecular biology (Cavicchioli et al. 2002). Applications of cold-adapted microorganisms include the construction of low-temperature protein expression systems by using these microorganisms as hosts to facilitate the overproduction of thermolabile proteins (Papa et al. 2007; Miyake et al. 2007). Extensive studies to understand the cold-adaptation mechanisms of these microorganisms are also being undertaken by many researchers (Cavicchioli 2006; D'Amico et al. 2006).

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J. Kawamoto · T. Kurihara (✉) · N. Esaki (✉)
Institute for Chemical Research,
Kyoto University, Uji, Kyoto 611-0011, Japan
e-mail: kurihara@scl.kyoto-u.ac.jp

N. Esaki
e-mail: esakin@scl.kyoto-u.ac.jp

M. Kitagawa · I. Kato
Takara Bio Inc, Seta 3-4-1, Otsu,
Shiga 520-9143, Japan

Recent studies of cold-adapted microorganisms revealed several strategies of these microorganisms to adapt to low temperatures, such as the production of cold-active enzymes (Feller and Gerday 2003; Siddiqui and Cavicchioli 2006), the modulation of the lipid composition to maintain the fluidity of the cell membrane (Chintalapati et al. 2004; Russell 1997), and the production of RNA chaperones to suppress the formation of undesired secondary structures of RNA (Hebraud and Potier 1999; Yamanaka et al. 1998). To obtain a better insight into microbial cold-adaptation mechanisms, genomic and proteomic approaches are being employed (D'Amico et al. 2006). Complete genomic DNA sequences are currently available for several cold-adapted microorganisms including *Desulfotalea psychrophila* LSv54 (Rabus et al. 2004), *Photobacterium profundum* SS9 (Vezzi et al. 2005), *Colwellia psychrerythraea* 34H (Methe et al. 2005), and *Pseudoalteromonas haloplanktis* TAC125 (Medigue et al. 2005). With the aid of these sequence data, it is possible to make a global identification of proteins produced under a particular growth condition. Because proteins that are inducibly produced at low temperatures are supposed to play important roles at low temperatures, the identification of cold-inducible proteins would provide clues for understanding the cold-adaptation mechanisms of microorganisms.

Shewanella livingstonensis Ac10, formerly called *Shewanella* sp. Ac10, is a cold-adapted bacterium isolated from Antarctic seawater (Kulakova et al. 1999). It was recently identified as a strain belonging to *S. livingstonensis* by Y. Nogi and C. Kato (Japan Agency for Marine-Earth Science and Technology) (unpublished). The strain grows well at low temperatures close to 0°C but does not grow at temperatures over 30°C (Kulakova et al. 1999). We have established transformation procedures, developed a heterologous protein expression system, and established targeted gene disruption methods for this bacterium (manuscript in preparation). In addition, we carried out whole genome sequencing of this bacterium and recently obtained the draft genome sequence. *Shewanella* species are widely distributed in various environments on the earth, and complete genomic DNA sequences of various *Shewanella* species have been determined [Genomes On-Line Database (http://www.genomesonline.org/)]. These include mesophilic *Shewanella oneidensis* MR-1 (Heidelberg et al. 2002) and piezophilic *Shewanella violacea* DSS12 (K. Nakasone, Kinki University, Higashihiroshima, Japan, personal communication). Comparative studies of these phylogenetically related strains that inhabit different environments would facilitate the understanding of the mechanisms of adaptation to various environments. In these respects, *S. livingstonensis* Ac10 is a fascinating model microorganism for the investigation of microbial cold-adaptation mechanisms.

In the present study, we performed proteomic studies of this bacterium to make a global identification of cold-inducible proteins and obtain clues to understand its cold-adaptation mechanism. We found that proteins involved in various cellular processes, such as the modulation of gene expression, motility, and membrane transport, were inducibly produced at low temperatures.

Materials and methods

Bacterial strain and culture conditions

Shewanella livingstonensis Ac10 isolated from Antarctic seawater was grown in 5 ml of Luria–Bertani (LB) medium (pH 7.0) for 48 h at 18°C, and then transferred to 300 ml of LB medium for further cultivation at 4°C and 18°C to the early stationary phase.

Proteome analysis

The cells grown at 4°C and 18°C were harvested by centrifugation and resuspended in 40 mM Tris–HCl (pH 7.0). The cell suspensions were sonicated and centrifuged. Proteins in the supernatants were used as soluble proteins for proteome analysis. Membrane proteins were extracted by using ReadyPrep Protein Extraction Kit (Membrane I) (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Proteins (300 μg) were loaded onto Immobiline DryStrip pH 4–7 (GE Healthcare UK Ltd, Buckinghamshire, UK), and isoelectric focusing was performed with PROTEAN IEF Cell (Bio-Rad Laboratories, Inc.) as recommended by the manufacturer. Treatment of the gel strips for the second-dimensional SDS-PAGE was carried out as described previously (Mineki et al. 2002). The second-dimensional SDS-PAGE was performed by using gradient gels with 10–20% acrylamide (PAG Large "Daiichi" 2D-10/20, Daiichi Pure Chemicals Co., Ltd, Tokyo, Japan). After fixation and staining with SYPRO Ruby (Invitrogen Corp., Carlsbad, CA, USA), the gels were scanned with an image analyzer, Typhoon 9400 (GE Healthcare UK Ltd). The expression pattern of proteins was analyzed using the image analysis software PDQuest ver. 7.0 (Bio-Rad Laboratories, Inc.). After scanning, the gels were restained with Negative Gel Stain MS Kit (Wako Pure Chemical Industries, Ltd, Osaka, Japan).

Gel spots for cold-inducible proteins whose relative abundance at 4°C was more than twofold that at 18°C were excised, and the proteins were digested with sequencing-grade modified trypsin (Promega Corporation, Madison, WI, USA). Peptide mass fingerprinting (PMF) analysis was performed by the standard method with Autoflex II MALDI-TOF systems (Bruker Daltonics, Billerica, MA, USA). Spectrum acquisition was ensured in a reflector mode with the following parameters: 42% laser power with a mass range of 180–4,460 Da and 5.3% detector gain. Calibration was performed using Peptide Calibration Standard (Bruker Daltonics). A local version of Mascot (Matrix Science Ltd., London, UK) was used to identify the proteins.

Quantitative real-time RT-PCR analysis

Total RNA was extracted with RNeasy Kit (QIAGEN Inc., Valencia, CA, USA) from cells cultivated at 4°C and 18°C. The RNA pellets were dissolved in 0.1% diethyl pyrocarbonate-treated water and stored at –80°C until use. Quantitative real-time RT-PCR was performed with SuperScript III Platinum SYBR Green One-Step qRT-PCR Kit (Invitrogen Corp.) and the Mx3000P Multiple Quantitative RT-PCR system (Stratagene, La Jolla, CA, USA). The threshold cycle (C_t) value for each sample was normalized with the C_t value for 16S rRNA.

Results

Growth profile of *S. livingstonensis* Ac10

Shewanella livingstonensis Ac10 grew with a doubling time of 8.8 h at 4°C, whereas the doubling time was 2.5 h at 18°C. No growth was observed at temperatures over 30°C. The yield of the cells grown at 4°C (9.2 g/l) was comparable to that of the cells grown at 18°C (8.0 g/l).

Global identification of cold-inducible proteins

Shewanella livingstonensis Ac10 was grown at 4°C and 18°C to the early stationary phase, and soluble and membrane proteins were prepared for two-dimensional gel electrophoresis (2DE) to identify cold-inducible proteins. Figure 1a shows the gel images obtained for the soluble proteins. Image analysis of the gels revealed that 91 spots were cold-inducible (more than a twofold increase at 4°C) (Fig. 1a, indicated by arrowheads). Cold induction was reproducibly observed for 48 of them in at least three sets of experiments (Fig. 1a, numbered spots), and further analysis was performed for these spots. The gel images for the membrane proteins are shown in Fig. 1b. Thirty-four membrane proteins were inducibly produced at 4°C (Fig. 1b, indicated by arrowheads), and reproducibility was confirmed for five of them (Fig. 1b, numbered spots).

The cold-inducible spots were excised from the gels and analyzed by PMF to identify the proteins. PMF analysis allowed the identification of 26 soluble proteins and two membrane proteins (Table 1). Twenty-two soluble proteins and three membrane proteins were not identified, probably due to the low abundance of the proteins or peptides ex-

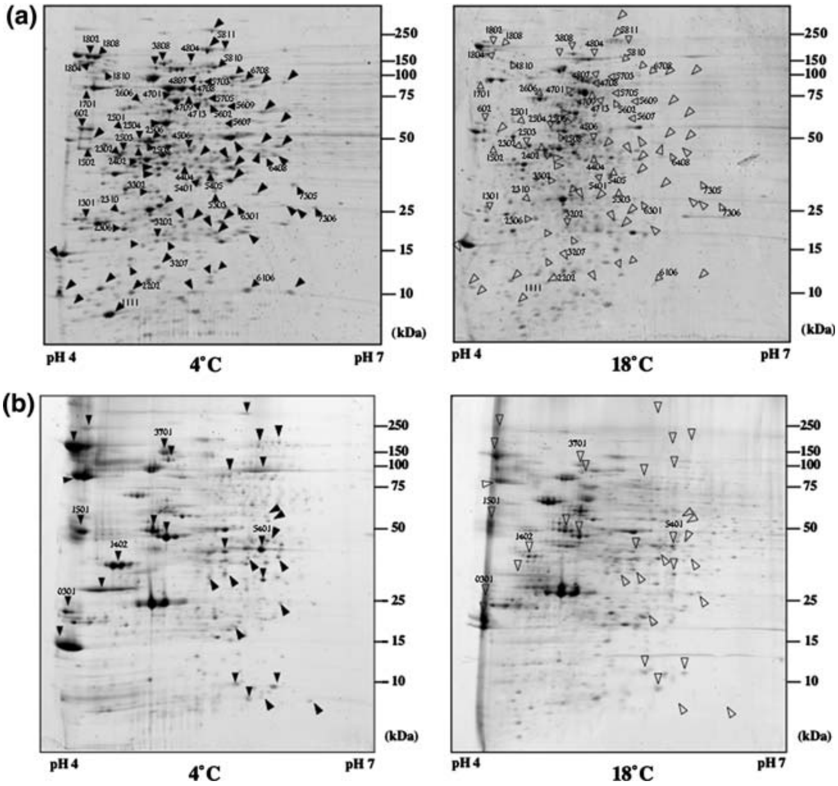

Fig. 1 Comparison of soluble (**a**) and membrane (**b**) proteins from *S. livingstonensis* Ac10 grown at 4°C (*left*) and 18°C (*right*). The gels were stained with SYPRO Ruby and analyzed with PDQuest ver. 7.0. The *arrowheads* indicate cold-inducible spots (91 and 34 spots for soluble and membrane proteins, respectively). Cold induction was reproducible for the numbered spots (48 and five spots for soluble and membrane proteins, respectively)

Table 1 Cold-inducible proteins of *S. livingstonensis* Ac10

Spot #	Gene	Protein[a]	*E*-value[b]	Molecular mass (kDa)[c]	p*I*[c]	Spot intensity (ppm) 4°C/18°C	Relative abundance (4°C/18°C)	Accession no.
Group 1. RNA synthesis and folding								
2508	*rpoA*	DNA-directed RNA polymerase α subunit	1.00E-161	36.1	4.64	782/<30	>26	AB284093
2402	*greA*	Transcription elongation factor	1.00E-58	17.4	4.65	2,591/<30	>86	AB284102
1111	*cspA*	Cold shock protein	7.00E-24	7.45	5.00	1,7315/<30	>580	AB284087
Group 2. Protein synthesis and folding								
7306	*tufB*	GTPase—translation elongation factor	0.00E+00	33.3	4.57	976/<30	>33	AB284113
3207	*efp*	Translation elongation factor P/translation initiation factor	2.00E-63	20.7	4.54	1,160/<30	>39	AB284108
5405	*tig*	FKBP-type peptidyl-prolyl *cis–trans* isomerase (trigger factor)	1.00E-174	54.2	4.85	4,790/858	5.6	AB284101
4404	*lysU*	Lysyl—tRNA synthase class II	0.00E+00	49.0	4.87	3,600/<30	>120	AB284089
Group 3. Membrane transport								
1402[d]	*ompC*	Porin	2.00E-06	37.7	4.46	2,1284/5,893	3.6	AB284090
5401[d]	*ompA*	Outer membrane protein A	1.00E-33	42.5	5.77	1,8325/<30	>610	AB284075
Group 4. Motility								
1810	*flgE*	Flagellar basal body and hook protein	3.00E-86	38.8	3.83	780/<30	>26	AB284085
602	*flgL*	Flagellin and related hook-associated protein	7.00E-27	19.3	3.99	5,016/159	32	AB284088
Group 5. Metabolism								
4506	*ppx1*	Inorganic pyrophosphatase/exopolyphosphatase	9.00E-83	33.6	4.49	1,027/<30	>34	AB284081
2504	*purD*	Phosphoribosylamine–glycine ligase	1.00E-166	45.3	4.66	1,348/<30	>45	AB284115
3202	*deoC*	Deoxyribose-phosphate aldolase	7.00E-79	27.7	4.55	1,872/<30	>62	AB284104
2306	*fixB*	Electron transfer flavoprotein α-subunit	2.00E-99	31.4	4.45	2,412/<30	>80	AB284109
3302	*nemA*	NADPH: flavin oxidoreductase	2.00E-83	24.3	4.31	1,550/<30	>52	AB284084
6408	*pdxJ*	Pyridoxal phosphate biosynthesis protein	3.00E-83	17.9	5.35	1,648/<30	>55	AB284100
1802	*ac10nuc*	Predicted extracellular nuclease	1.00E-160	93.6	4.26	6,058/<30	>200	AB284091
2503	*ac10cp*	Predicted carboxypeptidase	1.00E-125	41.9	4.42	915/<30	>31	AB284086
Group 6. Other functions								
4701	*ac10C417081*	6Fe-6S prismane cluster-containing protein	0.00E+00	60.3	4.55	988/<30	>33	AB284110
4709	*ftsZ*	Cell division GTPase	1.00E-122	40.4	4.57	1,155/<30	>39	AB284074
3808	*ac10omp*	Predicted outer membrane protein	0.00E+00	92.9	4.71	1,517/<30	>51	AB284079
1301	*tsx*	Nucleotide-binding outer membrane protein	5.00E-53	28.2	4.23	1,887/<30	>63	AB284082
1804	*ac10tn*	Transposase	2.00E-19	13.3	8.79	4,281/<30	>140	AB284094
Group 7. Unknown function								
2310	*ac10C130109*	–	–	21.8	4.65	755/<30	>25	AB284083
2202	*ac10C097028*	–	–	14.6	4.52	1,119/<30	>37	AB284078
1502	*ac10C346010*	–	–	11.4	9.33	2,394/<30	>80	AB284107
1701	*ac10C084124*	–	–	74.9	4.24	2,798/<30	>93	AB284077

[a] Proteins in the database showing the highest sequence similarity to the proteins from *S. livingstonensis* Ac10

[b] *E*-value is a parameter that describes the number of hits that can be expected to be identified by chance when searching a database of a particular size (http://www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html). The values listed indicate similarities between the proteins from *S. livingstonensis* Ac10 and their homologs from the database. A small value indicates high similarity

[c] Molecular mass and p*I* values were calculated by using the draft genome sequence of *S. livingstonensis* Ac10

[d] Proteins found in the membrane fraction

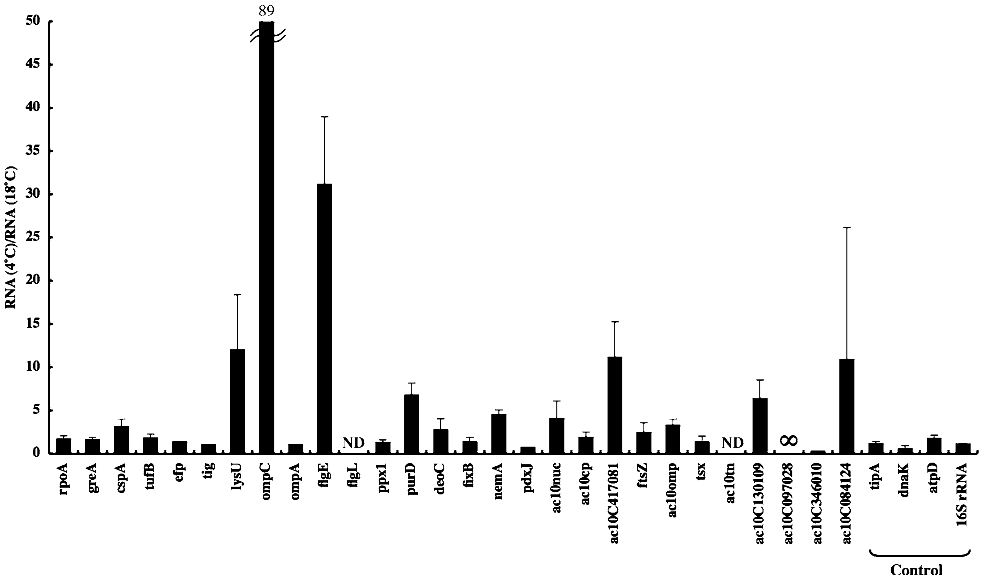

Fig. 2 Transcriptional levels of the genes coding for cold-inducible proteins. The ratios of the amounts of mRNA in the cells grown at 4°C and 18°C are indicated. All values were normalized with the ratio of the amounts of 16S rRNA in the cells grown at 4°C and 18°C. ND: No RT-PCR product was obtained for the cells grown at 4°C and 18°C. ∞: the RT-PCR product was obtained for the cells grown at 4°C but not for those grown at 18°C

tracted from the gels. The identified proteins can be classified into the following seven groups according to their sequence similarity to the proteins in the database: RNA synthesis and folding (group 1), protein synthesis and folding (group 2), membrane transport (group 3), motility (group 4), metabolism (group 5), other functions (group 6), and unknown function (group 7). The ratio of the spot intensity at 4°C to that at 18°C was more than 580 for CspA (Fig. 1a, no. 1111) and more than 610 for OmpA (Fig. 1b, no. 5401).

Analysis of the transcriptional levels of the genes coding for cold-inducible proteins

To determine whether the production of cold-inducible proteins is transcriptionally regulated, mRNAs for these proteins were quantified by real-time RT-PCR as described in Materials and methods. Because 2DE analysis showed that the spot intensities of TipA, DnaK, and ATP synthase β subunit (AtpD) were not significantly different at 4°C and 18°C (data not shown), mRNA coding for these proteins and 16S rRNA were used as controls. The results are summarized in Fig. 2.

The amounts of mRNA for the following 14 genes were more than two times larger at 4°C than at 18°C: *cspA*, *lysU*, *ompC*, *flgE*, *purD*, *deoC*, *nemA*, *ac10nuc*, *ac10C417081*, *ftsZ*, *ac10omp*, *ac10C130109*, *ac10C097028*, and *ac10C084124*. In contrast, the cultivation of the cells at 4°C did not significantly increase the transcription level of 12 genes, *rpoA*, *greA*, *tufB*, *efp*, *tig*, *ompA*, *ppx1*, *fixB*, *pdxJ*, *ac10cp*, *tsx*, and *ac10C346010*, although the spot intensities of the proteins encoded by these genes were increased at low temperatures (Table 1).

Discussion

Regulation of the expression of cold-inducible proteins

We identified 28 cold-inducible proteins of *S. livingstonensis* Ac10 by proteomic analysis of the cells grown at 4°C and 18°C (Table 1). These proteins are supposed to participate in various cellular processes, such as the modulation of gene expression, motility, and membrane transport. Analysis of the expression of the genes by real-time RT-PCR revealed that 14 of them were cold-inducible at the level of transcription (>twofold) (Fig. 2). It is noticeable that the degree of increase in the mRNA amount was less significant than that of the increase in the protein amount for most of these genes: the only two exceptions were *ompC* and *flgE*. The degree of increase in the amount of mRNA for *ompC* was more significant than that of increase in its protein amount. The results suggest that the expression of these genes, except for *flgE*, is regulated at both the transcription and post-transcription levels. We found 12 cold-inducible proteins whose expression was not up-regulated at 4°C at the level of transcription. Thus the regulation of expression of the cold-inducible proteins of *S. livingstonensis* Ac10 depends on various mechanisms.

Cold-inducible proteins involved in RNA and protein synthesis

The amounts of three proteins involved in RNA synthesis and folding (RpoA, GreA, and CspA) increased at 4°C (Table 1). RpoA is a subunit of RNA polymerase, which plays important roles in the assembly of RNA polymerase, promoter recognition by site-specific protein–DNA interaction, and transcriptional activation (Ebright and Busby 1995). GreA induces the cleavage and removal of the 3′ proximal dinucleotide from the nascent RNA to control transcriptional fidelity (Hogan et al. 2002). CspA is an RNA chaperone, which is supposed to make the secondary structure of mRNA suitable for translation (Jiang et al. 1997). These proteins may be required for the efficient and accurate transcription and proper folding of RNA at low temperatures, where base pairing of nucleic acids is stable and undesired secondary structures of RNA tend to form.

RNA polymerase is composed of RpoA, RpoB, RpoC and a sigma factor. Since we did not identify the spots for RNA polymerase subunits other than RpoA in this study, it is not clear whether the amounts of these proteins are increased at low temperatures. Inducible nature of the subunits other than RpoA will be studied to reveal whether the amount of functional RNA polymerase is increased at low temperatures.

CspA was identified as a major cold-shock protein in *Escherichia coli* (Yamanaka et al. 1998). CspA is supposed to play a central role in the cold-shock response in *E. coli*. In *S. livingstonensis* Ac10, however, CspA was produced in the stationary phase, suggesting that it is required for normal growth at low temperatures.

Tig of *S. livingstonensis* Ac10 was inducibly produced at 4°C (Table 1). It is a ribosome-associated chaperone with peptidyl-prolyl *cis–trans* isomerase (PPIase) activity and facilitates proper folding of newly synthesized proteins (Kramer et al. 2004). Cold induction of Tig may contribute to efficient translation at low temperatures. The protein consists of three domains, an N-terminal domain mediating association with ribosomes, a central substrate-binding domain with homology to FKBP proteins showing PPIase activity, and a C-terminal domain of unknown function. Suzuki et al. reported that an FKBP family member protein with PPIase activity (FKBP22) is inducibly produced at low temperatures in *Shewanella* sp. SIB1 (Suzuki et al. 2004). Although Tig of *S. livingstonensis* Ac10 is supposed to have PPIase activity, it is different from FKBP22 in that Tig has N-terminal and C-terminal domains that are not found in FKBP22. *S. livingstonensis* Ac10 has two copies of the genes coding for FKBP22 homologs, FkpA_C295011 and FkpA_C367122, showing 90 and 43% identities to FKBP22, respectively, but their expression was not confirmed in the present study.

Tig was first discovered in *E. coli* as a protein triggering the translocation of the precursor of pro-OmpA into a membrane vesicle (Crooke and Wickner 1987). pro-OmpA is a precursor of a major outer membrane protein, OmpA. We found that OmpA of *S. livingstonensis* Ac10 was inducibly produced at 4°C (Table 1). Cold-inducible Tig is possibly involved in the production of OmpA at low temperatures.

Translation elongation factors play a key role in protein synthesis on ribosomes and assist in the elongation of nascent polypeptide chains (Weijland et al. 1992). At low temperatures, protein synthesis is slowed because the activities of proteins involved in protein synthesis are suppressed. We found that putative elongation factors, TufB and Efp, which are supposed to act as carriers of aminoacyl-tRNAs, were inducibly produced at 4°C (Table 1). These proteins may enhance global protein synthesis at low temperatures.

Lysyl-tRNA synthetase, LysU (Freist and Gauss 1995), was identified as a cold-inducible protein (Table 1). Thus *S. livingstonensis* Ac10 possibly enhances the insertion of lysine into proteins at low temperatures. Many cold-adapted enzymes have a high Lys/Arg ratio in order to increase protein flexibility (Siddiqui and Cavicchioli 2006). LysU may contribute to the production of such lysine-rich cold-adapted enzymes.

Cold-inducible proteins involved in membrane transport

Two putative outer membrane porin homologs, OmpA and OmpC, were inducibly produced at 4°C (Table 1). Homologs of these proteins from other bacteria form channels for hydrophilic solutes and play important roles in the uptake of nutrients (Nikaido 2003). It is well known that bacteria regulate the production of their outer membrane porin depending on their environments. For example, *Shewanella frigidimarina* NCIMB400R inducibly produces an outer membrane porin, IfcO, under the control of the iron-responsive element under anaerobic conditions (Reyes-Ramirez et al. 2003). A deep-sea bacterium, *Photobacterium profundum* SS9, inducibly produces OmpL under high hydrostatic pressure conditions (Welch and Bartlett 1996). Thus these bacteria modulate the permeability of their outer membranes to adapt to their environments. Cold induction of OmpA and OmpC of *S. livingstonensis* Ac10 may counteract the low diffusion rate of solutes at low temperatures and enables the efficient uptake of nutrients.

Cold-inducible proteins involved in cell motility

The amounts of hook-related proteins of flagella, FlgE and FlgL (Homma et al. 1990), were increased at 4°C (Table 1). We found the consensus sequence of σ 54-dependent promoters and the gene coding for the σ 54-dependent transcriptional activator protein (Wosten 1998) in the upstream and downstream regions, respectively, of the gene cluster containing *flgE*. These factors possibly regulate the expression of *flgE*. Cold induction of FlgE and FlgL suggests that *S. livingstonensis* Ac10 forms different types of flagella depending on the cultivation temperature or produces flagella only at low temperatures to modulate its motility. To support this speculation, we found that the cells were more motile at 4°C than at 18°C. The physiological significance of higher motility at low temperatures remains to be clarified.

Cold-inducible FtsZ

FtsZ, which forms the division septum, is an essential protein for cell division highly conserved among prokaryotes (Addinall and Holland 2002). Most microorganisms have one copy of FtsZ, whereas *S. livingstonensis* Ac10 has two copies of the genes coding for FtsZ homologs, FtsZC26 and FtsZC19. FtsZC26 shows much higher similarity to other bacterial FtsZ than FtsZC19. We found that FtsZC26 was inducibly produced at 4°C (Table 1). The result raised the possibility that this bacterium changes its morphology depending on its growth temperature by altering the amount of the cell division protein. To support this speculation, we found that the cells were significantly shorter at 4°C than at 18°C.

Comparison of proteomes of various cold-adapted microorganisms

Proteomic studies have been conducted for several cold-adapted microorganisms such as *Methanococcoides burtonii* (Goodchild et al. 2004, 2005), *Bacillus psychrosaccharolyticus* (Seo et al. 2004), *Psychrobacter cryohalolentis* K5 (Bakermans et al. 2007), and *Psychrobacter articus* 273-4 (Zheng et al. 2007). It was found that different cold-adapted microorganisms produce different cold-inducible proteins. Thus different cold-adapted microorganisms are supposed to use different strategies to cope with cold environments. Nevertheless, some of those strategies appear to be common in several cold-adapted microorganisms. Cold induction of an RNA chaperone, CspA, is observed in many bacteria, including *Arthrobacter globiformis* SI55 (Berger et al. 1997), *P. cryohalolentis* K5 (Bakermans et al. 2007), and *S. livingstonensis* Ac10. PPIase, which facilitates protein folding, is inducibly produced at low temperatures in several cold-adapted microorganisms, such as *Shewanella* sp. SIB1 (Suzuki et al. 2004), *M. burtonii* (Goodchild et al. 2004), *P. articus* 273-4 (Zheng et al. 2007), and *S. livingstonensis* Ac10, although the type of cold-inducible PPIase is different in different cold-adapted microorganisms. It is likely that isomerization of peptidyl-prolyl bonds for proper protein folding is a crucial process for many microorganisms to survive at low temperatures. The modulation of RNA polymerase is also supposed to be important for the cells to grow at low temperatures because subunits of these complexes of several cold-adapted microorganisms, RNA polymerase subunit E from *M. burtonii* (Goodchild et al. 2004) and RNA polymerase α subunit from *P. articus* 273-4 (Zheng et al. 2007) and *S. livingstonensis* Ac10, are up-regulated at low temperatures. More distinctive features of proteomes of cold-adapted microorganisms will be clarified by future proteomic studies of other cold-adapted microorganisms, which will facilitate our understanding of their cold-adaptation mechanisms.

Cold adaptation is similar to pressure adaptation in several respects (Welch et al. 1993; Ishii et al. 2005). For example, both low temperature and high pressure decrease membrane fluidity, and the cells must modify the membrane component to maintain the membrane fluidity upon exposure to these environments. It has been reported that many cold shock proteins are inducibly synthesized under high-pressure conditions. Thus, characterization of cold-inducible proteins may also be helpful to understand mechanism of cellular adaptation to high pressure.

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References

Addinall SG, Holland B (2002) The tubulin ancestor, *ftsZ*, draughtsman, designer and driving force for bacterial cytokinesis. J Mol Biol 318:219–236

Bakermans C, Tollaksen SL, Giometti CS, Wilkerson C, Tiedje JM, Thomashow MF (2007) Proteomic analysis of *Psychrobacter cryohalolentis* K5 during growth at subzero temperatures. Extremophiles 11:343–354

Berger F, Normand P, Potier P (1997) *capA*, a *cspA*-like gene that encodes a cold acclimation protein in the psychrotrophic bacterium *Arthrobacter globiformis* SI55. J Bacteriol 179:5670–5676

Cavicchioli R (2006) Cold-adapted archaea. Nat Rev Microbiol 4:331–343

Cavicchioli R, Siddiqui KS, Andrews D, Sowers KR (2002) Low-temperature extremophiles and their applications. Curr Opin Biotechnol 13:253–261

Chintalapati S, Kiran MD, Shivaji S (2004) Role of membrane lipid fatty acids in cold adaptation. Cell Mol Biol (Noisy-le-grand) 50:631–642

Crooke E, Wickner W (1987) Trigger factor: a soluble protein that folds pro-OmpA into a membrane-assembly-competent form. Proc Natl Acad Sci USA 84:5216–5220

D'Amico S, Collins T, Marx JC, Feller G, Gerday C (2006) Psychrophilic microorganisms: challenges for life. EMBO Rep 7:385–389

Ebright RH, Busby S (1995) The *Escherichia coli* RNA polymerase α subunit: structure and function. Curr Opin Genet Dev 5:197–203

Feller G, Gerday C (2003) Psychrophilic enzymes: hot topics in cold adaptation. Nat Rev Microbiol 1:200–208

Freist W, Gauss DH (1995) Lysyl-tRNA synthetase. Biol Chem Hoppe Seyler 376:451–472

Goodchild A, Saunders NF, Ertan H, Raftery M, Guilhaus M, Curmi PM, Cavicchioli R (2004) A proteomic determination of cold adaptation in the Antarctic archaeon, *Methanococcoides burtonii*. Mol Microbiol 53:309–321

Goodchild A, Raftery M, Saunders NF, Guilhaus M, Cavicchioli R (2005) Cold adaptation of the antarctic archaeon, *Methanococcoides burtonii* assessed by proteomics using ICAT. J Proteome Res 4:473–480

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

Heidelberg JF, Paulsen IT, Nelson KE, Gaidos EJ, Nelson WC, Read TD, Eisen JA, Seshadri R, Ward N, Methe B, Clayton RA, Meyer T, Tsapin A, Scott J, Beanan M, Brinkac L, Daugherty S, DeBoy RT, Dodson RJ, Durkin AS, Haft DH, Kolonay JF, Madupu R, Peterson JD, Umayam LA, White O, Wolf AM, Vamathevan J, Weidman J, Impraim M, Lee K, Berry K, Lee C, Mueller J, Khouri H, Gill J, Utterback TR, McDonald LA, Feldblyum TV, Smith HO, Venter JC, Nealson KH, Fraser CM (2002) Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. Nat Biotechnol 20:1118–1123

Hogan BP, Hartsch T, Erie DA (2002) Transcript cleavage by *Thermus thermophilus* RNA polymerase. Effects of GreA and anti-GreA factors. J Biol Chem 277:967–975

Homma M, DeRosier DJ, Macnab RM (1990) Flagellar hook and hook-associated proteins of *Salmonella typhimurium* and their relationship to other axial components of the flagellum. J Mol Biol 213:819–832

Ishii A, Oshima T, Sato T, Nakasone K, Mori H, Kato C (2005) Analysis of hydrostatic pressure effects on transcription in *Escherichia coli* by DNA microarray procedure. Extremophiles 9:65–73

Jiang W, Hou Y, Inouye M (1997) CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. J Biol Chem 272:196–202

Kramer G, Rutkowska A, Wegrzyn RD, Patzelt H, Kurz TA, Merz F, Rauch T, Vorderwulbecke S, Deuerling E, Bukau B (2004) Functional dissection of *Escherichia coli* trigger factor: unraveling the function of individual domains. J Bacteriol 186:3777–3784

Kulakova L, Galkin A, Kurihara T, Yoshimura T, Esaki N (1999) Cold-active serine alkaline protease from the psychrotrophic bacterium *Shewanella* strain Ac10: gene cloning and enzyme purification and characterization. Appl Environ Microbiol 65:611–617

Medigue C, Krin E, Pascal G, Barbe V, Bernsel A, Bertin PN, Cheung F, Cruveiller S, D'Amico S, Duilio A, Fang G, Feller G, Ho C, Mangenot S, Marino G, Nilsson J, Parrilli E, Rocha EP, Rouy Z, Sekowska A, Tutino ML, Vallenet D, von Heijne G, Danchin A (2005) Coping with cold: the genome of the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. Genome Res 15:1325–1335

Methe BA, Nelson KE, Deming JW, Momen B, Melamud E, Zhang X, Moult J, Madupu R, Nelson WC, Dodson RJ, Brinkac LM, Daugherty SC, Durkin AS, DeBoy RT, Kolonay JF, Sullivan SA, Zhou L, Davidsen TM, Wu M, Huston AL, Lewis M, Weaver B, Weidman JF, Khouri H, Utterback TR, Feldblyum TV, Fraser CM (2005) The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. Proc Natl Acad Sci USA 102:10913–10918

Mineki R, Taka H, Fujimura T, Kikkawa M, Shindo N, Murayama K (2002) In situ alkylation with acrylamide for identification of cysteinyl residues in proteins during one- and two-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Proteomics 2:1672–1681

Miyake R, Kawamoto J, Wei Y, Kitagawa M, Kato I, Kurihara T, Esaki N (2007) Construction of a protein expression system operating at low temperatures using a cold-adapted bacterium, *Shewanella* sp. Ac10, as the host. Appl Environ Microbiol (in press)

Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67:593–656

Papa R, Rippa V, Sannia G, Marino G, Duilio A (2007) An effective cold inducible expression system developed in *Pseudoalteromonas haloplanktis* TAC125. J Biotechnol 127:199–210

Rabus R, Ruepp A, Frickey T, Rattei T, Fartmann B, Stark M, Bauer M, Zibat A, Lombardot T, Becker I, Amann J, Gellner K, Teeling H, Leuschner WD, Glockner FO, Lupas AN, Amann R, Klenk HP (2004) The genome of *Desulfotalea psychrophila*, a sulfate-reducing bacterium from permanently cold arctic sediments. Environ Microbiol 6:887–902

Reyes-Ramirez F, Dobbin P, Sawers G, Richardson DJ (2003) Characterization of transcriptional regulation of *Shewanella frigidimarina* Fe(III)-induced flavocytochrome c reveals a novel iron-responsive gene regulation system. J Bacteriol 185:4564–4571

Russell NJ (1997) Psychrophilic bacteria—molecular adaptations of membrane lipids. Comp Biochem Physiol A Physiol 118:489–493

Seo JB, Kim HS, Jung GY, Nam MH, Chung JH, Kim JY, Yoo JS, Kim CW, Kwon O (2004) Psychrophilicity of *Bacillus psychrosaccharolyticus*: a proteomic study. Proteomics 4:3654–3659

Siddiqui KS, Cavicchioli R (2006) Cold-adapted enzymes. Annu Rev Biochem 75:403–433

Suzuki Y, Haruki M, Takano K, Morikawa M, Kanaya S (2004) Possible involvement of an FKBP family member protein from a psychrotrophic bacterium *Shewanella* sp. SIB1 in cold-adaptation. Eur J Biochem 271:1372–1381

Vezzi A, Campanaro S, D'Angelo M, Simonato F, Vitulo N, Lauro FM, Cestaro A, Malacrida G, Simionati B, Cannata N, Romualdi C, Bartlett DH, Valle G (2005) Life at depth: *Photobacterium profundum* genome sequence and expression analysis. Science 307:1459–1461

Weijland A, Harmark K, Cool RH, Anborgh PH, Parmeggiani A (1992) Elongation factor Tu: a molecular switch in protein biosynthesis. Mol Microbiol 6:683–688

Welch TJ, Farewell A, Neidhardt FC, Bartlett DH (1993) Stress response of *Escherichia coli* to elevated hydrostatic pressure. J Bacteriol 175:7170–7177

Welch TJ, Bartlett DH (1996) Isolation and characterization of the structural gene for OmpL, a pressure-regulated porin-like protein from the deep-sea bacterium *Photobacterium* species strain SS9. J Bacteriol 178:5027–5031

Wosten MM (1998) Eubacterial sigma-factors. FEMS Microbiol Rev 22:127–150

Yamanaka K, Fang L, Inouye M (1998) The CspA family in *Escherichia coli*: multiple gene duplication for stress adaptation. Mol Microbiol 27:247–255

Zheng S, Ponder MA, Shih JY, Tiedje JM, Thomashow MF, Lubman DM (2007) A proteomic analysis of *Psychrobacter articus* 273–4 adaptation to low temperature and salinity using a 2-D liquid mapping approach. Electrophoresis 28:467–488