Extremophiles (2010) 14:171–183
DOI 10.1007/s00792-009-0296-5

ORIGINAL PAPER

Occurrence and distribution of *capB* in Antarctic microorganisms and study of its structure and regulation in the Antarctic biodegradative *Pseudomonas* sp. 30/3

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Received: 5 August 2009 / Accepted: 1 December 2009 / Published online: 20 December 2009

Abstract The analysis of the cold-shock domain (CSD)-encoding genes, *capB and cspA*, by PCR amplification showed presence of *capB* in all 18 Antarctic *Pseudomonas* isolates, but the absence of *cspA*. Nucleotide sequence analysis of *capB* ORF from a biodegradative *Pseudomonas* 30/3 and its regulatory sequences including the promoter and 5′-UTR was determined and compared with the other CSD-encoding genes. Expression analysis using translational gene fusion of the putative *capB* promoter and its flanking sequence from *Pseudomonas* sp. 30/3 with *lacZ*′ exhibited a significant increase in β-galactosidase activity at 15 and 6°C. Unlike the expression of *E. coli* CspA, *Pseudomonas* sp. 30/3 showed a slow but steady increase of the CapB expression at 6°C. Subcellular localization of CapB at 6°C showed accumulation in and around the nucleoid whereas at 22 or 30°C, it was identified around the nucleoid as well as in the cytosol. Our study attempts to elucidate the detailed structure of *capB* from *Pseudomonas* 30/3 and the role of 5′UTR in the transcriptional regulation along with the possible role of CapB in transcription and translation suited for the cold adaptation of this bacterium in Antarctic environment.

Keywords Cold adaptation · *capB* · Antarctic microorganism · *Pseudomonas* 30/3 · Immunolocalization

Communicated by A. Oren.

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Introduction

Microorganisms inhabiting the Antarctic environment exhibit adaptive features necessary to cope with extreme cold conditions. One of the adaptive responses characterized in Antarctic as well as in some of the mesophilic bacteria is the accumulation of proteins of the cold-shock domain (CSD) family and the regulation of their corresponding genes (Bej et al. 2000; Panicker et al. 2002; Phadtare et al. 1999; Schindler et al. 1999; Wouters et al. 2000; Yamanka et al. 1998). Two classes of small bacterial proteins that consist of a single nucleic acid-binding domain (CSD) have been described: (1) Csps or cold-shock proteins that are expressed immediately after temperature downshift (Goldstein et al. 1990; Graumann and Marahiel, 1996, 1998); and (2) Caps or cold acclimation proteins that are expressed during prolonged growth at cold temperatures (Jiang et al. 1993). CSPs have been extensively described in both Gram-negative and Gram-positive

bacterial species (Graumann and Marahiel 1998). A well-studied example is the *cspA* family of genes in *Escherichia coli*, which consist of nine homologs to the major cold-shock protein CspA (CS7.4) (Goldstein et al. 1990; Phadtare et al. 1999). And all nine individual members are differentially regulated in response to low temperature stress (Yamanka et al. 1998).

The CSD is an ancient and the evolutionary conserved nucleic acid-binding domain within prokaryotes and eukaryotes (Graumann and Marahiel 1998; Wolffe et al. 1992; Wolffe 1994). In eukaryotes, CSPs exist as a nucleic acid-binding domain within multidomain proteins, called CSD proteins (Somerville, 1999). Among the most widely studied eukaryotic CSD proteins is the Y-box transcription factor that contains an N-terminal domain, a CSD, and a C-terminal auxiliary domain. The functions of Y-box transcription factors have been studied in detail but none of these functions are directly related to cold-shock response or cold adaptation (Somerville, 1999).

The *E. coli* CspA consists of five β-barrel sheets with two consensus RNA-binding domains (RNP1 and RNP2) placed side by side on separate β-sheets (Newkirk et al. 1994; Schindelin et al. 1994). Similar structures have been observed in other bacterial CSPs and CspB from *Bacillus subtilis* (Schindelin et al. 1993). It has been reported that CspA functions as a RNA chaperone by minimizing the formation of secondary structures in mRNA and allowing efficient translation at low temperatures (Bae et al. 2000). The *cspA* promoter is constitutively expressed at 37°C though its activity is enhanced following cold shock of bacterial cultures (Tanabe et al. 1992; Fang et al. 1997). Many other CSPs have been found to have a similar function like that of CspA during the temperature down-shift (Bae et al. 2000). In contrast to *cspA*, which has been identified both in mesophilic and psychrotrophic microorganisms, the *cap*s have so far been identified only in cold-adapted bacteria (Fang et al. 1998; Berger et al. 1997; Gumley and Iniss 1996; Hebraud et al. 1994; Roberts and Inniss 1992). So far, only four *cap*s have been sequenced and characterized: *capA* and *capB* in *P. fragi* (Michel et al. 1997), *capA* from *Arthrobacter globiformis* (Berger et al. 1996, 1997) and the *capB* from *Pseudomonas* strain 30/3 (Panicker et al. 2002). These genes share 60–70% nucleotide identity with *cspA* open reading frame.

The expression of *cspA* is regulated in a complex manner at the transcriptional (Tanabe et al. 1992; Goldenberg et al. 1997; Mitta et al. 1997), mRNA stability (Brandi et al. 1996; Goldenberg et al. 1996; Fang et al. 1997) and translational levels (Mitta et al. 1997). The feature that contributes to the cold-shock induction of *cspA* and three other genes, *cspB*, *cspG* and *cspI,* is their long, highly homologous 5′ untranslated region (5′-UTR; 159 bases for *cspA*, 161 bases for *cspB*, 160 bases for *cspG* and 145 bases for *cspI*). They all have similar AT-rich region upstream of the −35 region called the UP element, which plays an important role in their transcription at low temperature (Mitta et al. 1997; Wang et al. 1999). The long 5′-UTRs of *cspA*, *cspB*, *cspG*, and *cspI* consists of a well-conserved motif, termed the cold box, which is believed to be involved in autoregulation at the end of the acclimation phase when exposed to cold temperatures (Jiang et al.1996; Fang et al. 1998).

In the present study, 35 mesophilic and psychrotrophic bacteria, primarily members of the *Enterobactericeae* family and 36 Antarctic bacterial isolates were screened for the presence of proteins of the cold-shock domain (CSD) family by PCR method using degenerate primer sets for *Enterobacteriaceae* and primer sets based on the related genes from *P. fragi*. *Pseudomonas* 30/3, a psychrotrophic bacterium belonging to the *Pseudomonas syringae* cluster (Panicker et al. 2002), was isolated from petroleum hydrocarbon (PAH)-contaminated soil from Wright Valley, Antarctica. In a previous study, we detected elevated expression of an 8-kDa protein in *Pseudomonas* 30/3 corresponding to the size of the CapB in *P. fragi* following exposure to 4°C but not at 15, 22, or 37°C (Panicker et al. 2002). In this study, the promoter region of the *capB* gene was cloned, sequenced and its activity was evaluated at 37, 15, and 6°C to determine its role in *capB* transcription at cold temperatures.

Materials and methods

Bacterial strains and media requirements

All Antarctic isolates (Table 1) were grown and maintained on R2A agar medium (Becton Dickenson, Franklin Lane, NJ) at 15°C. The mesophilic bacterial strains (Table 1) were cultured on nutrient agar (Becton Dickenson). The growth temperatures for all strains are listed in Table 1. *E. coli* strain NM522 [F′{*pro*AB$^+$, *lac*I^q, *lac*Z ΔM15}*sup*E, *thi*1 Δ(lac*pro*$^-$AB) Δ*hsd*5(r$^-$m$^-$)λ^-] was used for transformation and maintenance of the *capB* promoters segments cloned on the promoter probe vector pMLB1034.

Analysis of the upstream region of *capB* ORF

The TOPO-Walker^{TM} kit (Invitrogen, Carlsbad, CA, USA) was used to amplify the upstream sequences of the *capB* ORF using the oligonucleotide primers GSP1, GSP2 and GSP3 (Table 2). The resulting PCR amplified fragments were cloned onto pCR4-Topo^{TM} using the TOPO-TA^{TM} cloning kit (Invitrogen, Carlsbad, CA). The nucleotide sequence of the cloned gene fragments was determined using M13 forward or reverse primers and an ABI Prism automated DNA sequencer (Perkin Elmer, Norwalk, CT) at

Table 1 List of bacterial strains, site and temperature of their isolation, growth media, and amplification results of *cspA* and *capB* and CapB expression observed in this study

Strain	Location/source	Growth temperature (°C)	Growth substrate/media	PCR amplification		Western blot
				cspA	*capB*	*CapB*
Antarctic soil isolates						
Pseudomonas ant5	Scott Base	15	Naphthalene	–	+	+
Pseudomonas ant6	Scott Base	4	Naphthalene	–	+	+
Pseudomonas ant9	Scott Base	28	Naphthalene	–	+	+
Pseudomonas 6/170	Scott Base	4	Naphthalene	–	+	+
Pseudomonas 7/156	Scott Base	4	JP8 Jet Fuel	–	+	+
Pseudomonas 8/48	Scott Base	16	Naphthalene	–	+	+
Pseudomonas 19/1	Marble Point	16	JP8 Jet Fuel	–	+	+
Pseudomonas 30/1	Vanda Station	16	Naphthalene	–	+	+
Pseudomonas 30/2	Vanda Station	16	Naphthalene	–	+	+
Pseudomonas 30/3	Vanda Station	16	JP8 Jet Fuel	–	+	+
Pseudomonas 44/15	Scott Base	15	Degrades dodecane	–	+	+
Pseudomonas 44/47	Scott Base	15	Degrades dodecane, hexadecane, pristane and toluene	–	+	+
Pseudomonas 43/11	Scott Base	15	Degrades toluene	–	+	+
Pseudomonas 5.1	Marble Point	15	Nitrogen fixer	–	+	+
Pseudomonas 5A	Marble Point	15	Degrades aromatics	–	+	+
Pseudomonas 5B	Marble Point	15	Degrades hexane	–	+	+
Pseudomonas 43/43	Scott Base	15	NA	–	+	+
Pseudomonas 43/40	Scott Base	15	NA	–	+	+
Rhodococcus 43/2	Scott Base	15	Degrades alkane	–	–	ND
Rhodococcus 5/14	Scott Base	15	Degrades alkane	–	–	–
Rhodococcus CH3/2	Cape Hallet	15	Degrades alkane	–	–	ND
Rhodococcus CH1/1	Cape Hallet	15	Degrades alkane	–	–	ND
Sphingomonas 43/03	Scott Base	15	Degrades Aromatic	–	–	ND
Sphingomonas Ant 17	Scott Base	15	Degrades Aromatic	–	–	ND
Sphingomonas Ant 20	Scott Base	15	Degrades Aromatic	–	–	ND
Hymenobacter 34/31	Ross Island	4	R2A	–	–	ND
Nocardioides 35/13	Ross Island	4	R2A	–	–	ND
Frigobacterium 34/19	Ross Island	4	R2A	–	–	ND
Bacillus 34/9	Ross Island	10	R2A	–	–	ND
Sporosarcina	McMurdo Dry Valleys	10	R2A	–	–	ND
Flavobacterium Ant1-1	Schirmacher Oasis	15	R2A	–	–	ND
Frigoribacterium Ant-1-2-1	Schirmacher Oasis	15	R2A	–	–	ND
Arthrobacter Ant1-5-3	Schirmacher Oasis	15	R2A	–	–	ND
Flavobacterium Ant1-6	Schirmacher Oasis	15	R2A	–	–	ND
Janthinobacterium Ant5-2	Schirmacher Oasis	15	R2A	–	–	ND
Janthinobacterium Ant1-7-2	Schirmacher Oasis	15	R2A	–	–	ND
Other Psychrotolerant Bacteria						
Pseudomonas aeruginosa	Southern ocean, Antarctica	15	NA	–	–	ND
Micrococcus cryophilus ATCC 15174	Frozen fresh pork sausage	10	NA	–	–	ND
Aquaspirillum arcticum ATCC 49402	Sediment under snow and ice, Resolute, Northwest Territory, Canada	4	NA	–	–	ND

Table 1 continued

Strain	Location/source	Growth temperature (°C)	Growth substrate/media	PCR amplification		Western blot
				cspA	*capB*	*CapB*
Shewanella benthica ATCC 43991	Digestive tract of amphipod, *Scopelocheirus shellengi*, Puerto Rico Trench	4	NA	+	–	ND
Mesophilic bacteria						
Escherichia coli ATCC 25404	Migula, wild type strain	37	NA	+	–	ND
Escherichia coli ATCC 35401 (pathogenic)	Human feces	37	NA	+	–	ND
E. hermanii ATCC 35650	Swine tonsil	37	NA	+	–	ND
E. fergusoni ATCC 35469	Human feces	37	NA	+	–	ND
E. blattae ATCC 29907	Hindgut of cockraoch	37	NA	+	–	ND
E. vulneris ATCC 29943	Human wound	37	NA	+	–	ND
Shigella dysenteriae ATCC 29026	Human feces	37	NA	+	–	ND
S. boydii ATCC 9732	Bovine	37	NA	+	–	ND
S. sonnei ATCC 29930	WRAIR I virulent; Type strain	37	NA	+	–	ND
S. flexneri ATCC 9748	Not available	37	NA	+	–	ND
Citrobacter freundii ATCC 8090	Not available	37	NA	+	–	ND
Enterobacter cloacae ATCC 13047	Spinal fluid	37	NA	+	–	ND
E. aerogenes ATCC 13048	Sputum	37	NA	+	–	ND
Proteus vulgaris ATCC 13315	Not available	37	NA	+	–	ND
Klebsiella pneomoniae ATCC 13883	Not available	37	NA	+	–	ND
K. oxytoca ATCC 12833	FDA strain	37	NA	+	–	ND
Aeromonas hydrophila ATCC 7966	From a tin of milk with fishy odor	37	NA	+	–	ND
Vibrio cholera ATCC 11623	Classical biovar	37	NA	+	–	ND
Salmonella enteritidis Typhimurium ATCC 18585	Cucumber roots	37	NA	+	–	ND
Edwardsiella tarda ATCC 15947	Human feces	37	NA	+	–	ND
Arthrobacter citreus ATCC 11624	Not available	37	NA	+	–	ND
Listeria monocytogenes ATCC 19112	Spinal fluid	37	NA	+	–	ND
Kluyvera ascorbata ATCC 33433	Human sputum	37	NA	+	–	ND
Acinetobacter calocoateus ATCC 15308	Not available	37	NA	–	–	ND
Xanthomonas campsetris ATCC 11057	Plant	26	NA	–	–	ND
Deinococcus radiodurans ATCC 13939	Irradiated ground pork and beef	26	NA	–	–	ND
Pseudomonas putida ATCC 17484	Not available	26	NA	–	+	ND
P. aeruginosa PAO 1485	Not available	26	NA	–	–	ND
P. fluorescens ATCC 13525	Pre-filter tanks	26	NA	–	–	ND
P. cepacia ATCC 25416	Plant derived food stuff (onion)	26	NA	–	–	ND
P. paucimobilis	Biodegradative	26	NA	–	–	ND

ND Not determined

the UAB sequencing core facility (http://seqcore.uab.edu/). The sequences were then compared and aligned with the nucleotide sequence database in Genbank (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST). The promoter, RBS and ORF sequences were analyzed using Softberry (http://softberry.com/berry.phtml) and Geneious (http://www.geneious.com) software. Multiple sequence alignments were performed by T-Coffee software (Notredame et al. 2000).

PCR amplification

Genomic DNA was purified from all the isolates tested by the method described by Ausubel et al. (1987). The

Table 2 Oligonucleotide primers used in this study for PCR amplification of various segments of *Pseudomonas* 30/3 *capB*

Gene	Primer sequences (5′→3′)	Origin	Location	Reference
capB				
L-PfcapB150	cttcctgcgtcgcagttatc	*Pseudomonas* sp. 30/3	501–520	This study
R-PfcapB150/GSP1[a]	ttagataacttgaacttcctcag		728–750	
capB-GSP2	ttctttcaggctcttgaagc	*Pseudomonas* sp. 30/3	659–678	This study
capB-GSP3	tcatcgttgaaccacttaacgg		563–584	
L-capB515BamHI	c*ggatcc*cgatacaccgtaggaaatcactcgc[a]	*Pseudomonas* sp. 30/3	64–87	This study
R-capB13BamHI	c*ggatcc*cggttgaaccacttaacggtacca		558–579	
L-capBproBamHI	c*ggatcc*cgatctccaagaatttttcctgctc		453–475	
cspA				
CSPU5	cccgaattcggtahagtaaaatggttyaackc	Degenerate primers for *Enterobacteriaceae*	NA	Francis and Stewart (1997)
CSPU3	cccgaatccggttacgttascwgctkshggdcc			

GSP Gene specific primer; *NA* Not available

[a] The restriction endonuclease recognition sequences are italicized

oligonucleotide primers used for the detection of *capB* and *cspA* are listed in Table 2. Each PCR amplification was performed in a 25 µl reaction volume consisting of 1 µg of purified genomic DNA; 200 µM of each of the dNTPs; 1 µM of each of the oligonucleotide primers and 2.0 U AmpliTaq (Perkin Elmer, Walham, MA) DNA polymerase; and 1× PCR reaction buffer [10× buffer consisted of 300 mM Tris.Cl (pH 9.0), 75 mM $(NH_4)_2SO_4$ and 2 mM $MgCl_2$]. All PCR amplifications were performed in a GeneAmp PCR 2400 thermocycler (Perkin Elmer, Norwalk, CT) with 25 cycles of amplification steps each at 94°C for 1 min, 50°C for 1 min and 72°C for 2 min. For those isolates that were PCR negative, an annealing temperature of 45°C was also attempted.

Similarly, PCR was also carried out to amplify different fragments of the promoter region of *capB* using oligonucleotide primers with appropriate restriction endonuclease recognition sites flanked at the 5′ end to facilitate cloning onto the promoter probe vector pMLB1034 (Table 2). The PCR cycling and reaction conditions were same as above with the annealing temperature being 50°C.

Analysis of the promoter segments of *capB* on pMLB1034

Segments of the 5′-upstream DNA were PCR amplified using *capB*-specific L-capBproBamHI and R-capB13BamHI; and L-capB515BamHI and R-capB13BamH1 primer sets generating 136 and 515 bp amplicons, respectively. The amplified DNA fragments were then cloned on a promoter probe vector pMLB1034 to establish two translational fusion plasmids, pBGP136 and pBGP515. *E. coli* NM522 was transformed with these plasmid constructs and 3 white colonies with putative clones were randomly selected on LB agar plates supplemented with ampicillin (50µg/ml), 3-bromo-4-chloro-β-D-thiogalactosidase (X-gal) and isopropyl-beta-D-thiogalactopyranoside (IPTG). The selected white colonies were inoculated in LB broth and plasmid DNA extracted using the Qiagen mini-prep plasmid purification columns (Qiagen, Valencia, CA). The purified DNA from each putative clone was treated with the respective restriction endonuclease (New England Biolab, Beverly, MA) and separated in a 1% (w/v) agarose gel at 5 V/cm to determine the molecular sizes of the cloned DNA fragments.

β-galactosidase assay

E. coli NM522 harboring pBGP136 or pBGP515 plasmids were grown in 50 ml of LB supplemented with 1 mM IPTG at 37°C until the optical density at 600 nm reached 0.5. Aliquots (15 ml each) of the culture were transferred to 15 and 6°C. β-galactosidase assay (Miller 1972) was performed using 0.5 ml culture at 0 h (time before transfer to different temperatures), 1, 3, and 6 h after temperature downshift. The assay was done in triplicate to ensure the consistency of the results.

Western blot

An aliquot of the overnight culture of *Pseudomonas* sp. 30/3 grown at 19 ± 2°C (room temperature) was diluted 1:20 with 1:10 Trypticase Soy Broth (TSB) (Becton Dickenson) and grown at 19 ± 2°C (room temperature) on a rotary shaker set at 150 rpm until the optical density at 450 nm reached 0.2. The culture was aliquoted (50 ml) and transferred to 6, 15, or 30°C. At 0, 1, 3, and 6 h of incubation, an aliquot of the culture was removed and the bacteria were harvested by centrifugation (10,000×*g* for 10 min). The cells were resuspended in PBS (pH 7.2) and the cell membrane disrupted by sonication on ice for 3–5 s.

The lysate was resuspended in 50 mM Tris–HCl buffer (pH 6.8) consisting of 2% (w/v) SDS, 10% (v/v) glycerol, 6% (v/v) 2-mercaptoethanol and 0.002% (v/v) bromophenol blue to 0.25 μg total protein/μl, and boiled for 5 min. The concentration of total protein was determined colorimetrically using the BCA Protein Assay Kit (Pierce, Rockford, IL) with bovine serum albumin (Sigma–Aldrich, St. Louis, MO) as a standard. These samples (2.5 μg total protein) were electrophoresed on a 15% (w/v) polyacrylamide gel and electrophoretically transferred onto polyvinyliden difluoride membranes (Millipore, Bedford, MA). For anti-CapB antiserum, first *capB* from *Pseudomonas* sp. 30/3 was PCR amplified and cloned into pET24 (b) plasmid (Novagen, EMD Chemicals, Madison, WI). The CapB protein was expressed by IPTG induction and then purified using the His.Tag kit (Novagen, EMD Chemicals). The antiserum for the CapB protein was developed in NZW rabbit with 3 boosts (14, 21 and 49 days) and 2 test-bleeds (35 and 56 days) (Cocalico Biologicals, Inc., Reamstoen, PA). The effective dilution of the anti-CapB rabbit-antiserum for western blot analysis was determined by reaction with the purified CapB protein and serial dilution of the antibody followed by a standard curve analysis (Ausubel et al. 1987). The membrane with immobilized proteins from *Pseudomonas* sp. 30/3 was incubated with rabbit anti-CapB antiserum (1:1000 dilutions) for 2 h at 22°C. Bound antibodies were detected with goat anti-rabbit peroxidase conjugated IgG (1:5000 dilution, Pierce), and the peroxidase activity was visualized using 0.02% (v/v) of 3, 3′-diaminobenzidine tetrahydrochloride (Pierce) in 0.1 M PBS (pH 6.2) containing 0.06% H_2O_2 (Thermo Fisher Scientific). The membrane was scanned and the relative amount of CapB expressed was analyzed with Scion Image software (http://www.scopncorp.com). The consistency of the results was determined from four individual assays. Similarly, western blot assays were performed for CapB expression in other Antarctic *Pseudomonas* sp. at 15°C (Table 1).

In situ localization of CapB by immunofluorescence staining

Immunofluorescence staining was performed by a modified method described by Harry et al. (1995). *Pseudomonas* 30/3 culture was grown in 1:10 v/v TSB media till exponential phase at 22°C and then aliquots of 50 ml each were subjected to 6 (cold shock), 22, and 30°C, respectively for 1 h.

Fixation and permeabilization of cells

The cells were vortexed to disrupt any clumps of bacteria. A 0.25-ml volume of bacterial culture was fixed with fixative solution at a final concentration of 2.4% (v/v) paraformaldehyde, 0.04% (v/v) glutaraldehyde, in 30 mM Na-PO_4 buffer (pH 7.5) for 10 min at room temperature and 50 min on ice. The fixed bacteria were washed thrice in PBS (pH 7.4) at room temperature and then resuspended in 90–125 μl of GTE (50 mM glucose, 20 mM Tris–HCl [pH 7.5], 10 mM EDTA). A freshly prepared lysozyme solution in GTE was added to a final concentration of 2 mg/ml. 10 μl of the fixed cells were dropped on a microscopic slide which was treated with 0.1% (wt/v) poly-L-lysine (Sigma, St. Louis, MO). After 30 s, the liquid was aspirated from the slides, and allowed to dry completely. The slides were dipped in −20°C methanol for 5 min and then in −20°C acetone for 30 s and again allowed to dry. A 10-μl volume of blocking solution (2% bovine serum albumin [BSA] in PBS, pH 7.4 [BSA-PBS]) was then added to the fixed cells, and the slides were then incubated for 15 min at room temperature.

Immunofluorescence staining of cells

The samples on the slides were incubated with rabbit anti-CapB antiserum (1:1500 dilutions in BSA-PBS) for 1 h at 22°C and were washed 10 times with PBS. The slides were then incubated with 7.5 mg/ml solution of the HiLyte Fluor 488-labeled goat anti-rabbit IgG secondary antibody (AnaSpec, San Jose, CA) in BSA–PBS for 1 h at 22°C in the dark. DAPI (Sigma, St. Louis, MO) was added with the secondary antibody at a final concentration of 0.01 mg/ml. The slides were washed 10 times with PBS and then once with mounting medium. Slides were mounted in PBS-glycerol solution and observed under a Leica fluorescence microscope (Bannockburn, IL) or stored at −20°C for several days before observation.

Results

Occurrence of the *cspA* and the *capB* genes that code for the CSD proteins

The results of the PCR amplification of *cspA* and *capB* in various microbial isolates are presented in Table 1. All 18 Antarctic *Pseudomonas* soil isolates exhibited 248 bp *capB* amplicon. Only 1 non-Antarctic strain, *P. putida* ATCC 17484 exhibited positive amplification. The *capB* in *Pseudomonas* sp. 30/3 was used as the positive control in this study (Panicker et al. 2002). These 18 Antarctic *Pseudomonas* isolates exhibited positive PCR amplification with the *capB* primers (L-capB515BamH1 or LcapBpro-BamH1 in combination with RcapB13BamH1), but were negative for *cspA* when amplified with the *cspA* gene-specific CSPU5 and CSPU3 universal primers. All *Enterobactericeae* and a few other mesophilic isolates

tested in this study showed positive amplification of a 200-bp *cspA* ORF with the *cspA* gene-specific universal primers. An Antarctic marine psychrophile, *S. benthica* ATCC 43992 was also positive for *cspA,* but was negative for *capB*. *P. putida* ATCC 17484 was the only mesophilic isolate that exhibited positive amplification of *capB* in this study. All other mesophilic *Pseudomonas* spp. were negative for both *cspA* and *capB*.

Structure of CapB and sequence comparison among other CSD proteins

CapB of *Pseudomonas* 30/3 is highly identical to CapB of *P. fragi* (98.6%). When the amino acid sequence of CapB was compared to other known cold-inducible CSPs in different bacteria (i.e., CspA, CspB, CspG and CspI from *E.coli* and CspB, CspC and CspD from *B.subtilis*), the amino acid identity/similarity was between 51.4 and 60.9%/60–71% (Fig. 1b). The three-dimensional structure of CspA has already been determined. It consists of five antiparallel β-strands forming a β-barrel structure with two β-sheets (Feng et al. 1998; Newkirk et al. 1994; Schindelin et al. 1994). CapB consists of well-conserved hydrophobic residues V9, I21, V30 (L29 in CapB), V32 (V31 in CapB), and V51 (Fig. 1b) which form a hydrophobic core in CspA. In addition, CapB also consists of two well-conserved RNA binding motifs, RNP1 and RNP2 (Fig. 1b). These facts suggest that like CspA, the CapB may form a similar structure to that of CspA and may also bind to RNA and single-stranded DNA.

Transcriptional regulation of *capB* expression

Upstream nucleotide sequences of the *capB* ORF

The BLAST (http://www.ncbi.nlm.nih.gov) nucleotide sequence comparison analysis of the 540-bp region upstream of the ATG of *capB* of *Pseudomonas* 30/3 exhibited 93% nucleotide identity with the upstream sequence of the *capB* gene in *P. fragi*. The nucleotide sequence information of *Pseudomonas* 30/3 is elaborated in Fig. 1a and the GenBank accession number is AF363392 (gi:13625472). The putative promoter sequences −35 (5′-TTGGCA-3′), −10 (5′-GGTTAAGGT-3′) were identified. The putative transcription initiation site is 3-bp downstream from the −10 promoter region (Fig. 1a). A typical RBS (5′-AGGA$_{7_9}$*ATG*) and an ORF of 210 bp between ATG and TAA were also identified (Fig. 1a). Thirteen base-pairs downstream from the transcription initiation nucleotide and within the 149 bp long 5′-UTR is a sequence with high identity to eubacterial cold-box elements (Figs. 1a, 2a). In *Pseudomonas* 30/3 *capB*, 7 out of 11 nucleotides are identical to the cold-box sequences of *E.coli cspA*, *cspB* and *cspG* (Fig. 2a).The level of identity exceeds that for the cold-box sequence from the *E. coli cspI* gene, which has only three to five nucleotides in common with other cold-box elements. And the level of identity is less than that for the cold-box sequence from *Anabaena crhC* and *M. burtonii deaD* (RNA helicases) which have seven to nine nucleotides in common with other cold-box elements (Fig. 2a). As shown in Fig. 2b, *capB* also contains downstream box (DB) downstream of translation initiation codon which has 10 out of 14 identical nucleotides to DB of *E. coli cspA*, the level of identity of DB sequence from *Pseudomonas* 30/3 *capB* exceeds that of DB from Anabaena *crhC* (Fig. 2b). Farther downstream in the 5′-UTR of *Pseudomonas* 30/3 *capB*, there is a putative 12-bases upstream sequence, which may be similar to upstream sequence from other cold-shock genes. Interestingly, *Pseudomonas* 30/3 *capB* does not have an AT-rich UP element upstream of the −35 promoter sequence, which plays an important role in the transcription at low temperature in *E.coli cspA, cspB, cspG* and *cspI* (Mitta et al. 1997; Wang et al. 1999).

Cold shock-inducible expression of *capB*

The pBGP136 construct consisting of a 127-bp DNA fragment excluding the cold box and promoter but including the *capB* RBS and the codons for the first 13 amino acid residues exhibited nearly insignificant (<30 Miller units) β–galactosidase activity at all temperatures (Fig. 3a, b). However, within this narrow range of β-galactosidase activity, cultures exposed to 6°C showed maximum expression with an increasing trend throughout the 6-h incubation period. The cultures exposed to 15°C showed a slight decline in the activity, whereas cultures at 37°C showed the least activity (<5 Miller unit) and lower than the activity measured at initial time (15 Miller unit).

The pBGP515 construct consisting of a 517-bp DNA fragment included in pGBP136 along with an additional 390 bp further 5′ DNA sequence upstream including entire 149 bp 5′UTR with cold box and *capB* promoter, showed significant increase (>1000 fold) in β–galactosidase activity at cold temperatures (Fig. 3c, d). Cultures exposed to 15°C for 1 h showed an increased activity of 3000 Miller units, eventually reaching a maximum activity of >4000 Miller units at 3 h and then declined to approximately 3000 Miller units at 6 h. In contrast, cultures at 6°C exhibited a steady increase in the expression of β-galactosidase ranging from 1000 Miller units after 1 h of incubation to >3000 Miller units at 6 h. However, the cultures exposed to 37°C during the entire 6-h time period exhibited a decline in activity after the initial 1 h of incubation.

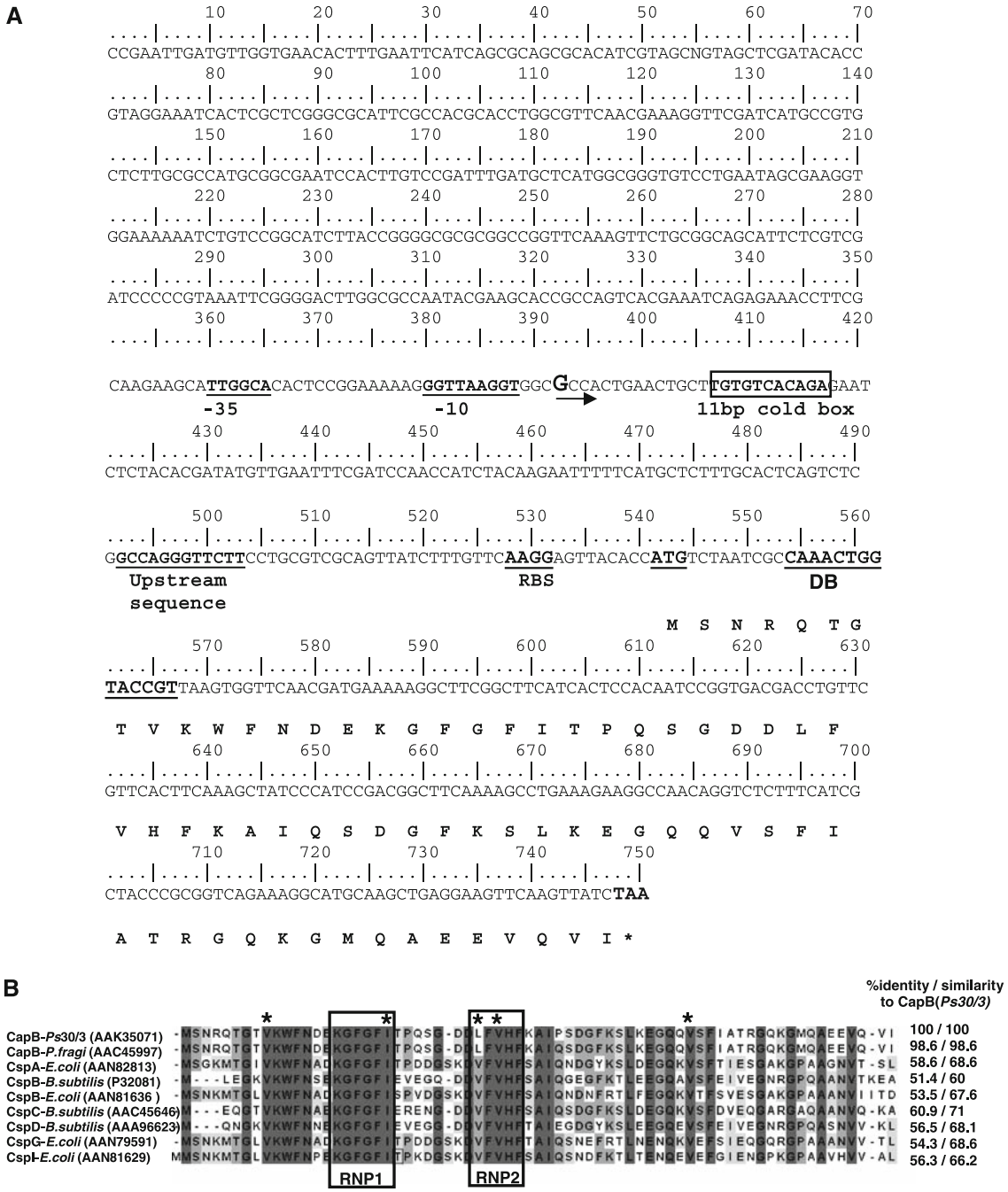

Fig. 1 **a** Nucleotide sequence of the 540-bp upstream region of the *capB* gene along with the 210-bp *capB* ORF from *Pseudomonas* sp.30/3. The putative promoter elements –35 and –10 regions as well as the ribosome binding site (RBS), upstream sequence and downstream box (DB) sequences are *underlined* and labeled. GenBank accession number is AF363392. The putative transcription start site is in *bold letters* and is marked by an *arrow*. The translation start codon ATGs are also in *bold letters* and are *underlined*. The putative 11-bp cold-box element is labeled and boxed. **b** Amino acid sequence alignments of CapB-*Ps*30/3 (database accession. no. AAK35071), CapB-*P.fragi* (AAC45997), CspA-*E.coli* (AAN82813), CspB *B.subtilis* (P32081), CspB-*E.coli* (AAN81636), CspC-*B.subtilis* (AAC45646), CspD *B.subtilis* (AAA96623), CspG-*E.coli* (AAN79591), CspI-*E.coli* (AAN81629). Alignments were performed by T-Coffee (Notredame et al. 2000). Their amino acid sequence identities/similarities are shown on the *right*, with CapB-*Ps*30/3 set at 100%. The residues forming the hydrophobic core in the *β*-barrel structure are indicated by *asterisks* above the sequences. The RNA binding motifs, RNP1 and RNP2, are *boxed*

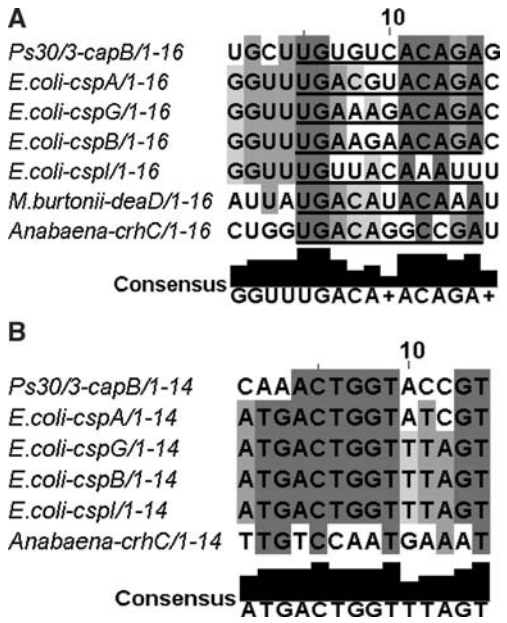

Fig. 2 **a** Comparison of cold-box elements from coldshock genes of *E. coli*, *Anabaena* and deaD gene from *M. burtonii* with the putative cold-box element from *Pseudomonas* 30/3 *capB* gene. Alignments were performed by T-Coffee (Notredame et al. 2000). 11-bp cold-box element is underlined. **b** Comparison of the downstream box (DB) sequence from coldshock genes of *E. coli* and *Anabaena* with the 14-bp putative DB from *Pseudomonas* 30/3 *capB* gene. Alignments were performed by T-Coffee (Notredame et al. 2000)

CapB expression

Western blot results exhibited elevated expression of CapB in *Pseudomonas* 30/3 cultures when exposed to 15 and 6°C, whereas the cultures exposed at 30°C exhibited progressively decreased expression from the initial time of incubation (Fig. 4). The expression of CapB continued to increase at a steady level at 6°C, whereas the level decreased slightly for cultures exposed at 15°C. Moreover, all Antarctic *Pseudomonas* sp. which exhibited positive amplification of capB also showed expression of CapB by western blot (Table 1).

In situ immunolocalization of CapB in *Pseudomonas* 30/3

In order to understand the possible cellular role of CapB in *Pseudomonas* 30/3, immunofluorescence staining was used to localize this protein using the anti-CapB rabbit-antiserum at different temperatures. The cellular location of the nucleoid was confirmed by DAPI staining (Fig. 5a). At 6°C, a dense accumulation of the anti-CapB antibody immunoconjugated with the green Hilyte Fluor 488-labeled goat anti-rabbit IgG secondary antibody was observed around the nucleoid region (Fig. 5b). At 22 or 30°C, the green fluorescence was dispersed in the cytosol as well as in the nucleoid region (Fig. 5c, d, e, f). In addition, cultures exposed at 6°C exhibited compact nucleoid as compared to the cultures exposed to 22 or 30°C.

Discussion

Psychrotrophic microorganisms demonstrate growth at a wide range of temperatures as high as 25°C or above and remain metabolically active at or below 0°C (Pikuta and Hoover 2007). Cold inducible proteins collectively known as cold-adaptive proteins, and the well-studied cold-shock proteins have been implicated in microbial adaptation to cold temperatures (Hebraud and Potier 1999). The cold adaptive gene, *capB* is present in cold-tolerant *Pseudomonas* spp. such as *P. fragi* K1, *P. syringae*, *P. fluorescens*, *P. tolaasii*, *P. solanacearum* (Hebraud et al. 1993). Similarly, we have shown in this study that *capB* is also present in all of the Antarctic biodegradative *Pseudomonas* spp. tested (Table 1).

We have investigated the role of the 5′-UTR and the cold-box sequences in the regulation of the *Pseudomonas* 30/3 *capB* gene. High level of β-galactosidase activity by the pBGP515 construct at cold temperatures suggested that the 515-bp untranslated DNA segment including the entire 149-bp 5′UTR with cold box and *capB* promoter along with the 13 amino acid residues including the downstream box sequence of the CapB in *Pseudomonas* 30/3 are required for the regulation of the *capB* and sustained expression of the CapB at cold temperatures. Unlike the expression of the CspA family of proteins that tends to exhibit transient expression immediately following downshift of the temperature (Etchegaray et al.1996), the CapB exhibited a steady increase following exposure to cold temperatures. This is similar to the previously described characteristic expression of the Caps during prolonged exposure to cold temperatures (Thieringer et al. 1998; Whyte and Inniss 1992). Therefore, it is possible that the unique and different nucleotide sequences on the 5′ upstream region of the *capB* ORF in *Pseudomonas* 30/3 and the absence of AT-rich UP element may be contributing to the regulation of this gene leading to the sustained expression of the CapB at cold temperatures. The sustained expression of CapB may be necessary for the adaptation of microorganisms in Antarctic perennially cold temperature environment. It has been shown that there is striking high structural similarity in four *csp* genes from *E. coli* and cold induced genes encoding DEAD-box RNA helicases from *E. coli*, *Anabaena* and *M. burtonii* (Lim et al. 2000). And all of the 5′-UTRs are greater than 100 bp in length including *Pseudomonas* 30/3 *capB* gene. *E. coli cspA*, *cspB*, and *cspG*, all have a downstream box (DB) located downstream of the translation initiation codon, which has

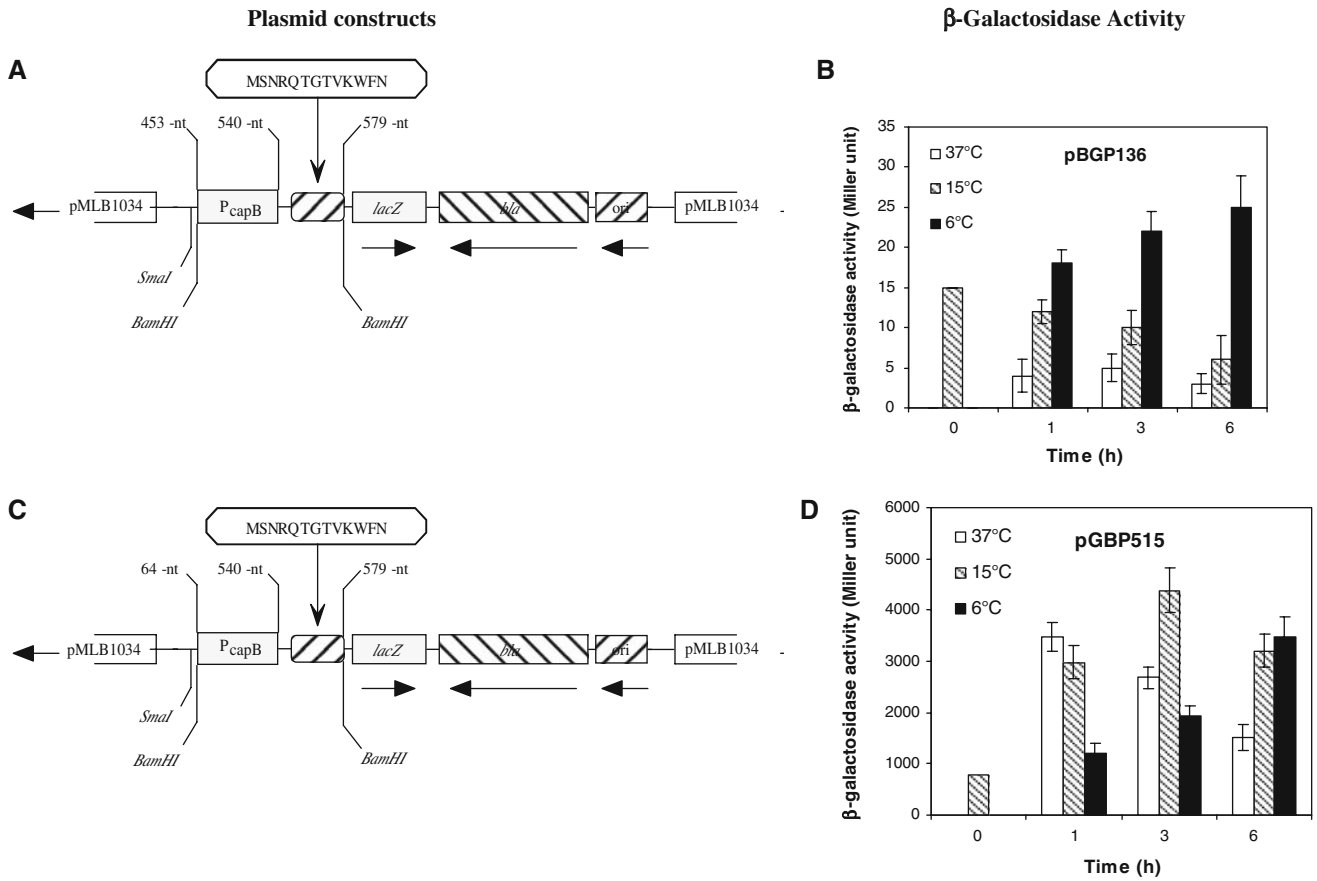

Fig. 3 **a** Construction of plasmid pBGP136. A translational fusion was constructed with the PCR amplified *capB* promoter consisting of the −35, −10, and the ribosome binding sequence and the sequences for the first 13 amino acid residues of the *capB* ORF on pMLB1034. **b** The results of the *β*-galactosidase assay exhibited by the pBGP136 construct. See text for the detail description of the results; **c** Construction of plasmid pBGP515. A translational fusion was constructed with the PCR amplified *capB* promoter consisting of the 476-bp upstream of the −35 and stretching up to the sequence for the first 13 amino acid residues of the *capB* ORF on pMLB1034; **d** The results of the *β*-galactosidase assay exhibited by the pBGP515 construct. See text for the detail description of the results

been shown to play an important role in cold-shock induction at the level of translation (Mitta et al. 1997). *Pseudomonas* 30/3 *capB* gene also have similar DB sequence (Fig. 2b) and a construct pGP476 consisting of entire 149 bp 5′UTR with cold box and *capB* promoter but without codons for the first 13 amino acid residues, i.e., without the DB sequence, did not show any *β*-galactosidase activity at 37 or 15°C and showed a low level of expression after the culture was exposed to 6°C for 3 h (data not shown). In this respect, it is similar to the *cspA* promoter that also requires the first 13 amino acid residues containing the DB sequence to enhance transcription of this gene and translation of the *cspA* mRNA at cold temperatures (Mitta et al. 1997). The cold-box sequences are well conserved, and except *E. coli cspI* sequence, at least 6 of the 11 nucleotides are in common between any one cold-box sequences (Fig. 2a). It has also been observed that the optimal temperature ranges for induction of the four *E. coli* cold-shock induced *csp* genes (*cspA, B, G* and *I*) are not same. Upon cold shock, CspA can be induced for broader range of temperature than that of CspB and CspG, which are restricted to lower and narrower temperature range (Etchegaray et al.1996). It has been suggested by the authors that specific sequence differences in the 5′-UTR and cold-box elements resulting in different mRNA secondary structures may play important roles in regulation. But authors from the same laboratory concluded that deleting the cold-box had little effect on cold-shock induction of *β*-galactosidase activity, and that instead a region 11 bp upstream of the ribosome binding site was important for translational efficiency of gene expression (Yamanka et al. 1999). Although all these reports indicate involvement of 5′-UTR in the regulation, the exact genetic structures and precise mechanisms for the function of the CSD-encoding genes at cold temperatures are rather complex and yet to be defined.

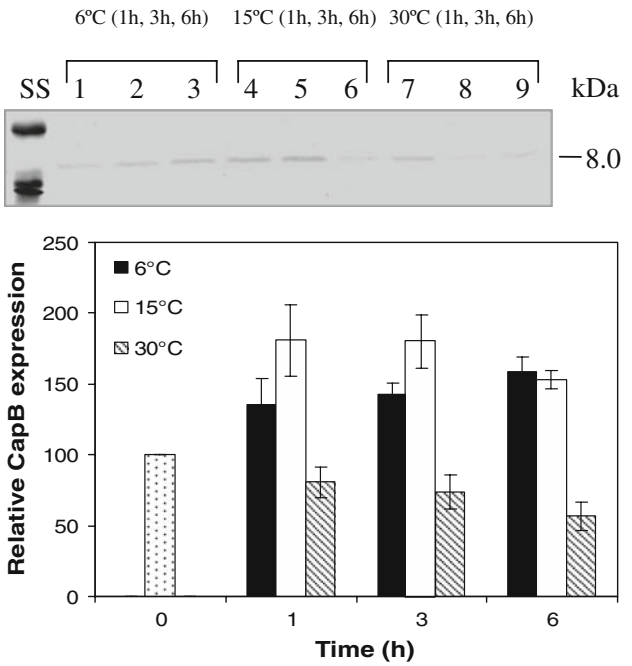

Fig. 4 Western blot analysis of the expression of CapB protein in *Pseudomonas* 30/3 following exposure to various temperatures. *Lanes 1–3* Cultures exposed to 6°C for 1, 3, and 6 h following treatment, respectively; *Lanes 4–6* cultures exposed to 15°C for 1, 3, and 6 h following treatment, respectively; *Lanes 7–9* cultures exposed to 30°C for 1, 3, and 6 h following treatment, respectively; *SS* Size Standard

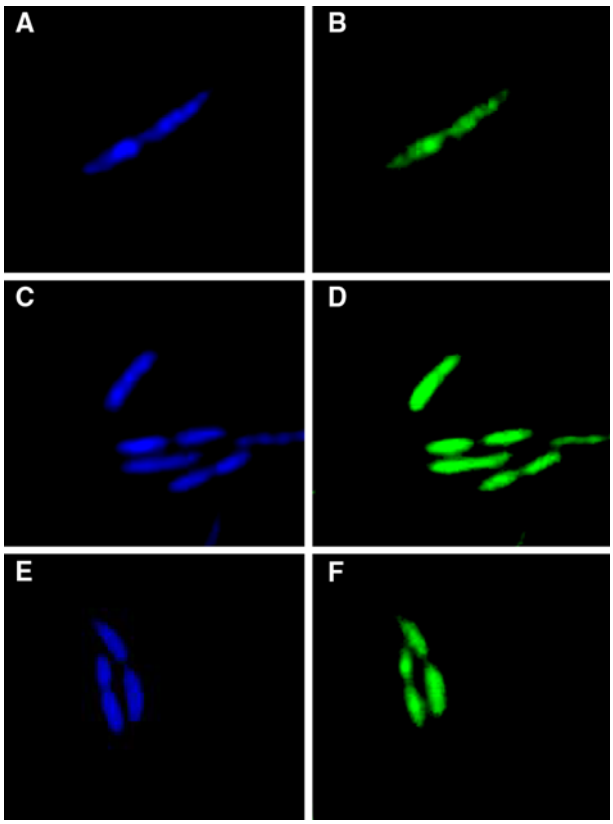

Fig. 5 Immunolocalization of CapB in *Pseudomonas* 30/3 at various temperatures; **a**, **c**, **e** The DAPI stained (*blue fluorescence*) nuclei at 6, 22, and 30°C, respectively; **b**, **d**, **f** The CapB bound HiLyte Fluor 488 (*green fluorescence*) at 6, 22, and 30°C, respectively. CapB can be seen localized around the compact nucleoid region at 6°C (**b**) and CapB localized both around nucleoid and cytosol at 22 and 30°C (**d**, **f**)

Also, we have investigated the possible in situ function of CapB by intracellular localization study. The amino acid sequence alignment among the *Pseudomonas* 30/3 CapB and other CSPs from different bacteria exhibited a relatively high sequence similarity in the conserved RNA binding motifs (RNP1 and RNP2) suggesting that *Pseudomonas* 30/3 CapB may have the same cellular function (Fig. 1b). In spite of the conserved RNP1 and RNP2 motifs, our study reveals that there are differences in the subcellular localization of *Pseudomonas* 30/3 CapB and CspA in *E. coli*. CspA in *E. coli* occupies a polar position away from the nucleoid at 37°C and maintains its position when subjected to cold shock (Giangrossi et al. 2001), whereas the CapB was localized in and around the nucleoid during cold shock. *Pseudomonas* 30/3 cells exhibited a more compact nucleoid at 6°C, which was with the zone of localization of CapB in the cytosolic spaces surrounding the nucleoid region. It has been reported that during cold shock, there is a decrease in the transcriptional and translational capacity of the cells leading to chromosome compaction in *B. subtilis* (Weber et al. 2001). Also, previous studies in both *E. coli* and *B. subtilis* have shown that the ribosomal proteins localize in a manner similar to CSPs, while RNA polymerase subunits and the primary sigma factor localize mainly in nucleoids (Azam et al. 2000; Lewis et al. 2000). During cold shock, CapB was localized mostly in the nucleoid region, which suggests similar localization as RNA polymerase and it implies a possible role for CapB in transcription. Whereas, at higher temperatures (22 and 30°C), CapB localizes as found for the ribosomal proteins suggesting that it functions at the same cellular location as ribosomes during translation. Therefore, the results from this study suggest that CapB has a possible role in both transcription and translation in *Pseudomonas* 30/3.

We have shown that the *capB* gene is conserved in Antarctic biodegradative *Pseudomonas* sp. Although the *capB* and CSD-encoding genes share common genetic features, the unique regulatory segment of a biodegradative *Pseudomonas* 30/3 *capB* gene could be responsible for the sustained expression of CapB protein. Moreover, the in situ localization of the CapB indicated that this protein has both transcriptional and translational regulatory role in this bacterium. The continuous expression of the CapB protein and its regulatory role in the transcription and translation of the essential genes may be necessary for this bacterium and

possibly other Antarctic *Pseudomonas* sp. tested in this study for survival in Antarctic perennially cold temperature environment.

Acknowledgments This study was supported in part the UAB Faculty Development award and the Department of Biology, and the Foundation of Research, Science and Technology, New Zealand (C09X0018). The logistic support was provided by Antarctica New Zealand; 2008 Tawani International Scientific Expedition (Tawani Foundation, Chicago, IL); and Antarctic *Maitri* (NCAOR, India) and *Novolazarevskaya* (Russia) stations. We thank Col (Ret) James Pritzker for supporting the Tawani Expedition; Rasik Ravindra (Director, NCAOR, India), Cdr. Arun Chaturvedi, Cdr. Pradip Malhotra and Ashit Swain for field support. We thank Edward Phillips at UAB High Resolution Imaging Shared Facility for the fluorescent microscopy study.

References

Ausubel FM, Brent R, Kingston RE, Moore DD, Smith JG, Sideman JG, Struhl K (eds) (1987) Current protocols in molecular biology. John Wiley & Sons, Inc., New York, pp 2.10–2.11

Azam TA, Hiraga S, Ishihama A (2000) Two types of localization of the DNA-binding proteins within the *Escherichia coli* nucleoid. Genes Cells 5:613–626

Bae W, Xia B, Inouye M, Severinov K (2000) *Escherichia coli* CspA-family RNA chaperones are transcription antiterminators. Proc Natl Acad Sci USA 97:7784–7789

Bej AK, Saul D, Aislabie J (2000) Cold tolerant alkane-degrading Rhodococcus species from Antartica. Polar Biol 23:100–105

Berger F, Morellet N, Menu F, Potier P (1996) Cold shock and cold acclimation proteins in the psychrotrophic bacterium *Arthrobacter globiformis* S155. J Bacteriol 178:2999–3007

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

Brandi A, Pietroni P, Gulazeri CO, Pon CL (1996) Post-transcriptional regulation of CspA expression in *Escherichia coli*. Mol Microbiol 19:231–240

Etchegaray JP, Jones PG, Inouye M (1996) Differential thermoregulation of two highly homologous cold-shock genes, *cspA* and *cspB*, of *Escherichia coli*. Genes Cells 1:171–178

Fang L, Jiang W, Bae W, Inouye M (1997) Promoter-independent cold-shock induction of *cspA* and its derepression at 37°C by mRNA stabilization. Mol Microbiol 23:355–364

Fang L, Hou Y, Inouye M (1998) Role of the cold-box region in the 5′ untranslated region of the *cspA* mRNA in its transient expression at low temperature in *Escherichia coli*. J Bacteriol 180:90–95

Feng W, Tejero R, Zimmerman DE, Inouye M, Montelione GT (1998) Solution NMR structure and backbone dynamics of the major cold shock protein (CspA) from *Escherichia coli*: evidence for conformational dynamics in the single-stranded RNA-binding site. Biochemistry 37:10881–10896

Francis KP, Stewart GS (1997) Detection and speciation of bacteria through PCR using universal major cold-shock protein primer oligomers. J Indust Microbiol Biotechnol 19:286–293

Giangrossi M, Exley RM, Le Hegarat F, Pon CL (2001) Different in vivo localization of the *Escherichia coli* proteins CspD and CspA. FEMS Microbiol Lett 202(2):171–176

Goldenberg D, Azar I, Oppenheim AB (1996) Differential mRNA stability of the *cspA* gene in the cold-shock response of *Escherichia coli*. Mol Microbiol 19:241–248

Goldenberg D, Azar I, Oppenheim AB, Brandi A, Pon CL, Gualerzi CO (1997) Role of *Escherichia coli cspA* promoter sequences and adaptation of translational apparatus in the cold shock response. Mol Gen Genet 256:282–290

Goldstein J, Pollit NS, Inouye M (1990) Major cold shock protein of *Escherichia coli*. Proc Natl Acad Sci USA 87:283–287

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

Graumann PL, Marahiel MA (1998) A superfamily of proteins that contain the cold-shock domain. Trends Biochem Sci 23:286–290

Gumley AW, Iniss WE (1996) Cold shock proteins and cold acclimation proteins in the psychrotrophic bacterium *Pseudomonas putida* Q5 and its transconjugants. Can J Microbiol 42:798–803

Harry EJ, Pogliano K, Losick R (1995) Use of immunofluorescence to visualize cell-specific gene expression during sporulation in *Bacillus subtilis*. J Bacteriol 177(12):3386–3389

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

Hebraud M, Garry P, Labadie J (1993) Ubiquity of low molecular mass cold-shock proteins. Abstracts of the fourth international symposium on *pseudomonas*, p 59

Hebraud M, Dubois E, Potier P, Labadie J (1994) Effect of growth temperatures on the protein levels in a psychrotrophic bacterium, *Pseudomonas fragi*. J Bacteriol 176:4017–4024

Jiang W, Jones P, Inouye M (1993) Chloramphenicol induced the transcription of the major cold-shock gene of *Escherichia coli*, *cspA*. J Bacteriol 175:5824–5828

Jiang W, Fang L, Inouye M (1996) The role of the 5′-end untranslated region of the mRNA for CspA, the major cold-shock protein of *Escherichia coli*, in cold-shock adaptation. J Bacteriol 178(16): 4919–4925

Lewis PJ, Thaker SD, Errington J (2000) Compartmentalization of transcription and translation in *Bacillus subtilis*. EMBO J 19:710–718

Lim J, Thomas T, Cavicchioli R (2000) Low temperature regulated DEAD-box RNA helicase from the Antarctic archaeon, *Methanococcoides burtonii*. J Mol Biol 297:553–567

Michel V, Lehoux I, Depret G, Anglade P, Labadie J, Hebraud M (1997) The cold shock response of the psychrotrophic bacterium *Pseudomonas fragi* involves four low-molecular-mass nucleic acid-binding proteins. J Bacteriol 179:7331–7342

Miller JH (1972) Experiments in molecular genetics. Cold Spring Laboratory, Cold Spring Harbor, NY, pp 352–355

Mitta M, Fang L, Inouye M (1997) Deletion analysis of *cspA* of *Escherichia coli*: requirement of the AT-rich UP element for *cspA* transcription and the downstream box in the coding region for its cold shock induction. Mol Microbiol 26:321–335

Newkirk K, Feng W, Jiang W, Tejero R, Emerson SD, Inouye M, Montelione GT (1994) Solution NMR structure of the major cold shock protein (CspA) from *Escherichia coli*: identification of a binding epitope for DNA. Proc Natl Acad Sci USA 91:5114–5118

Notredame C, Higgins DG, Heringa J (2000) T-Coffee: a novel method for fast and accurate multiple sequence alignment. J Mol Biol 302:205–217

Panicker G, Aislabie J, Saul D, Bej A (2002) Cold tolerance of *Pseudomonas* sp. 30/3 isolated from oil-contaminated soil, Antarctica. Pol Biol 225:5–11

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

Pikuta EV, Hoover RB (2007) Microbial extremophiles at the limits of life. Crit Rev Microbiol 33:183–209

Roberts ME, Inniss WE (1992) The synthesis of cold shock proteins and cold acclimation proteins in the psychrophilic bacterium *Aquaspirillum articum*. Curr Microbiol 25:275–278

Schindelin H, Marahiel MA, Heinemann U (1993) Universal nucleic acid-binding domain revealed by crystal structure of the *B. subtilis* major cold-shock protein. Nature 364:164–168

Schindelin H, Jiang W, Inouye M, Heinemann U (1994) Crystal structure of CspA, the major cold shock protein of *Escherichia coli*. Proc Natl Acad Sci USA 91:5119–5123

Schindler T, Graumann PL, Perl D, Ma S, Schmid FX, Marahiel MA (1999) The family of cold shock proteins of *Bacillus subtilis*. Stability and dynamics in vitro and in vivo. J Biol Chem 274:3407–3413

Somerville J (1999) Activities of cold-shock domain proteins in translation control. Bioessays 21:319–325

Tanabe H, Goldstein J, Yang M, Inouye M (1992) Identification of the promoter region of the *Escherichia coli* major cold shock gene, *cspA*. J Bacteriol 174:3867–3873

Thieringer HA, Jones PG, Inouye M (1998) Cold shock adaptation. BioEssays 20:49–57

Wang N, Yamanka K, Inouye M (1999) CspI, the ninth member of the CspA family of *Escherichia coli*, is induced upon cold shock. J Bacteriol 181:1603–1609

Weber MHW, Volkov AV, Fricke I, Marahiel MA, Graumann PL (2001) Localization of cold shock proteins to cytosolic spaces surrounding nucleoids in *Bacillus subtilis* depends on active transcription. J Bacteriol 183(21):6435–6443

Whyte LG, Inniss WE (1992) Cold shock proteins and cold acclimation proteins in a psychrotrophic bacterium. Can J Microbiol 38:1281–1285

Wolffe AP (1994) Structural and functional properties of the evolutionarily ancient Y-box family of nucleic acid binding proteins. Bioessays 16:245–251

Wolffe AP, Tafuri S, Ranjan M, Familari M (1992) The Y-box factors: a family of nucleic acid binding proteins conserved from *Escherichia coli* to man. New Biol 4:290–298

Wouters JA, Rombouts FM, Kuipers OP, de Vos WM, Abee T (2000) The role of cold-shock proteins in low-temperature adaptation of food-related bacteria. Syst Appl Microbiol 23:165–173

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

Yamanka K, Mitta M, Inouye M (1999) Mutation analysis of the 5′ untranslated region of the cold shock *cspA* mRNA of *Escherichia coli*. J Bacteriol 181:6284–6291