Extremophiles (2012) 16:167–176
DOI 10.1007/s00792-011-0423-y

REVIEW

Bacterial gene expression at low temperatures

J. T. Trevors · A. K. Bej · N. Mojib · J. D. van Elsas · L. Van Overbeek

Received: 30 June 2011 / Accepted: 13 December 2011 / Published online: 3 January 2012
© Springer 2011

Abstract Under suboptimal environmental conditions such as low temperatures, many bacteria have an extended lag phase, altered cell structures, and composition such as a less fluid (more rigid) and leaky cytoplasmic membrane. As a result, cells may die, enter into a starvation mode of metabolism or a physiologically viable but non-culturable (VBNC) state. In the latter state, the amount of gene expression per cell is virtually undetectable. In this article, gene expression under (suboptimal) low temperature conditions in non-psychrophilic environmental bacteria is examined. The pros and cons of some of the molecular methodologies for gene expression analysis are also discussed.

Keywords Cold adaptation · Antifreeze protein · qRT-PCR · Microarray · 2-D protein gel · SYTO Green · Caps · Csps · AFP · IBP · Antarctica · Bacteria · Extreme · Environment

Communicated by H. Atomi.

J. T. Trevors (✉)
Laboratory of Microbiology, School of Environmental Sciences, Rm. 3220 Bovey Bldg., University of Guelph, Guelph, ON N1G 2W1, Canada
e-mail: jtrevors@uoguelph.ca

A. K. Bej (✉) · N. Mojib
Department of Biology, 464 Campbell Hall, University of Alabama at Birmingham, Birmingham, AL 35294, USA
e-mail: abej@uab.edu

N. Mojib
e-mail: naziamoj@uab.edu

J. D. van Elsas (✉)
Department of Microbial Ecology, CEES, University of Groningen, Nijenborgh 4, Groningen, The Netherlands
e-mail: J.D.van.Elsas@rug.nl

L. Van Overbeek (✉)
Wageningen University and Research Centre, Plant Research International, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
e-mail: l.s.vanoverbeek@wur.nl

Introduction

For decades, the activities of a specific bacterial species, either in a community or as a single population, have been investigated on the basis of physiology/biochemistry obtained in the laboratory and, to a lesser extent, in a field setup. In the natural environment, bacteria are exposed to many complex physico-chemical factors (van Elsas et al. 2007). Under such changing environmental conditions, bacteria respond via changes in their gene expression, to maintain cellular functions.

About 80% of the Earth's surface temperature is 15°C or less (Rodrigues and Tiedje 2008). In this temperature range, bacteria that thrive are either psychrophilic or psychrotrophic; such bacteria are generally considered to be cold-adapted. Thus, low temperature climates provide unique environments in which cold-tolerant or cold-adapted microorganisms coordinate their gene expression to drive environmentally adapted cell functions. Moreover, even for thermophilic bacteria, temperatures suboptimal for growth can be considered to represent "cold" environments. For example, temperatures of about 20°C can be suboptimal for some mesophiles and 40–50°C for thermophiles. For most bacterial species, we lack an understanding of which genes or gene sets are expressed during sustained low temperatures or temperature downshifts. We

also do not understand the time required for the onset of expression of genes that become induced, the duration of expression, and the substrate concentrations required to coordinate the gene expression in the fluctuating environment to maintain the steady-state physiological conditions. This provides the opportunity for us to better research and understand microbial processes that are modulated by the fluctuations of optimal to suboptimal (low) growth temperatures.

The development of molecular technologies has advanced our understanding as to how bacteria survive, grow and divide under low and subzero temperatures. However, there is a paucity of knowledge on low temperature gene expression in many bacterial species under diverse and often rapidly or seasonally changing environmental conditions. Additional challenges are the choice of gene classification groups useful in bacterial gene expression analysis and the applications of the technological advances (microarray, reverse-transcription quantitative real-time polymerase chain reaction, 2-D protein gel electrophoresis followed by analysis of protein digests with nano-liquid chromatography–MS/MS) and the advantages/limitations of the approaches (Table 1). In this article, we examine bacterial gene expression at low temperatures in pure cultures and selected environmental samples along with the methods and challenges/limitations involved in discerning gene regulatory systems under such conditions.

Table 1 Challenges and limitations with determining bacterial gene expression at low temperatures

Biological limitations	1. Extended generation times
	2. Viable but non-culturable cells (VBNC)
	3. Damaged or injured cells
	4. Bacterial mRNAs are used immediately as templates while being transcribed
	5. Mutated genes
Technological limitations	1. Unknown mRNA extraction efficiencies
	2. mRNA turnover is rapid in cells (minutes)
	3. mRNA rapidly degraded during extraction
	4. Unknown gene expression time scales
	5. Variable gene expression per cell
	6. Randomness in samples
	7. Primer design to capture randomness in microbial populations
	8. Suboptimal environmental conditions
	9. 2-D gel electrophoresis do not resolve all proteins
	10. Unknown genes and functions
Data analysis limitations	1. Establishing correlations between gene expression and activity
	2. Analyses of simultaneous multiple genes expressions and presentation of quantitative results
	3. Expression of gene activity on a per cell basis
	4. Sampling plans for environmental samples

Methods and challenges/limitations in bacterial gene expression analysis

Reverse-transcription quantitative real-time polymerase chain reaction (RT-qPCR)

RT-qPCR is a sensitive method for detection and quantification of mRNA transcripts (Saleh-Lakha et al. 2008, 2009a, b, 2011). The technique uses total purified RNA, which is reverse transcribed to produce cDNA copies as templates in a PCR amplification, often with probes (Taqman probes) designed to identify the gene(s) of interest. Fluorescent technology is used to quantify the product at the end of each amplification cycle, where the fluorescent signal is correlated to the amount of product at the end of each PCR cycle when Taqman probe is not used. qPCR with the fluorescently-labeled Taqman probes uses the logarithmic phase of product accumulation (rather than the end-point abundance of PCR product), which provides a more accurate estimate of the transcripts. Specific primers used in the amplification reactions may not capture the sequence diversity present in environmental populations/communities. PCR inhibitors such as humic acids, clays, metal ions and enzyme inhibitors that are often co-extracted with RNA may interfere with the PCR amplification. In addition, RNA extracts from environmental samples (e.g., soil, decomposing plant and animal litter, sediment, ice, water) may not represent optimum coverage of the inhabiting bacterial population. However, qPCR is sensitive (low target detection limits), specific to the targeted RNA, reproducible and allows quantitation over 6 orders of magnitude (Saleh-Lakha et al. 2011).

Microarrays

Microarrays are tools for the simultaneous analysis of the function of multiple genes (or all genes in a bacterial genome), providing an immense data set on the physiology of the bacterial cells under different environmental conditions (Dubois et al. 2003). The specificity of the probe–target association depends on the degree of sequence divergence, which can be variable among environmental samples. Although specificity can be optimized with the targeted cDNA/RNA and the probe hybridization conditions, it is a significant factor in the detection of the sequence divergence of related species in environmental samples. Microarrays are specific, sensitive, accurate, reproducible, have a wide applicability and allow for the study of global gene expression or total cell physiology.

Limitations include the exclusion of sequence–divergent species and various contaminants interfering with the hybridization signals.

2-D protein gel electrophoresis

In two-dimensional protein gel electrophoresis, bacterial cells are lysed, the proteins solubilized and the total protein concentrations usually determined by the Bradford method. In the first dimension, the extracted proteins are separated using immobilized pH gradient gel strips (IPG, linear pH 4–7). In the second dimension, the proteins are separated based upon the molecular weights and normally the gels stained with Sypro Ruby fluorescent dye (Molecular Probes, Eugene, Oregon). The gel images are collected using FluorS gel imager (e.g., BioRad, Hercules, CA or equivalent device). Occasionally, the gels are stained with silver nitrate to facilitate the excision of the protein spots followed by proteolytic digests and subsequent composition analysis. The resulting peptides are analyzed by nano-liquid chromatography–MS/MS (Mykytczuk et al. 2011). The MS/MS spectra are acquired on doubly, triply and quadruply charged ions. Proteins can be identified and grouped according to their automatic annotation by functional roles using the TIGR-CMR database (http://cmr.tigr.org/cgi-bin/CMR/GenomePage.cgi?org=gtf). The percentage of proteins in a given functional category can be scored against the total number of proteins identified in the sample. Analysis of hypothetical proteins, identified from the 2-D gels, can be performed using BLASTp, which allows us to find potential homologues in other bacterial species. For protein sequences that do not match and cannot be assigned to known protein identities, the mass spectral data can be analyzed using de novo sequencing program PEAKS (Bioinformatics Solutions Inc, Waterloo, ON, Canada). The resulting peptide sequences are matched to the complete NCBI database. Automated peptide matches can be verified individually. The genes can be aligned and examined for gene clusters that may represent putative operons. Operon predictions can be made using the operonHMM software program (Bergman et al. 2007).

Challenges and limitations in determining bacterial gene expression at low temperatures are summarized in Table 1. Some of the challenges are technical and include unknown extraction efficiencies and stability (half-lives) of transcripts, the presence of PCR inhibitors, correct primer design, detection limits of transcripts and the interpretation of the results from methodologies such as microarray or RT-qPCR. In addition, the duration of gene expression and numbers of transcripts are unknown for most genes under diverse, temporal and spatial environmental conditions. Moreover, interpretation of gene expression data is challenging when cells in pure cultures or environmental samples are at different stages in their cell cycle (period from one cell division to the next). Unsynchronized or unbalanced cell growth means that the data arising from the gene expression analysis are averages of gene expression from all cells that were lysed and from which mRNA was extracted in the samples. Although an average value can be obtained, accurate information on the amount of gene expression per cell (active, moribund or just died) is a challenge.

Gene expression

Bacterial survival and growth at low temperatures even by psychrotrophic and psychrophilic bacteria presents a challenge to the cells confronted with environmental entropy, and their responses via differential gene expression. The genetic programming in the cells instructs them how to grow and divide if the conditions are within the environmental range that permits the cells to synthesize new cytoplasm, replicate the genome and divide. The other alternatives for cells are to form cysts or spores if the microorganism is a spore-former, die, or enter a viable but non-culturable (VBNC) physiological state. This occurs when nutrients are limited along with other unfavorable environmental conditions and cell division is not possible (Fig. 1). Some examples of gene expression with low temperature as an environmental condition are given below.

An excellent study by Rodrigues and Tiedje (2008) concluded that a strain of *Exiguobacterium sibiricum* isolated from a depth of about 44 m in a three million-year-old Siberian permafrost sample exhibited little differential gene expression between 4 and 28°C. The authors concluded this microorganism was constitutively adapted to cold temperatures. This conclusion was based on the finding that at both 4 and 10°C, genes related to temperature stress were not differentially expressed as would have been observed in mesophilic microorganisms.

Ducey et al. (2010) characterized a microbial community capable of nitrification at low temperatures. Nitrification, the biological oxidation of ammonia to nitrite and then nitrate is of fundamental importance in the global nitrogen cycle. They reported high rates of nitrification at temperatures from 5 to 20°C in a lagoon. This demonstrated the expression of the nitrification genes in different species over a temperature range. The actual amount of expression per cell and the duration of expression are not known.

Pseudomonas mandelii in liquid cultures were researched to assess the effect of temperature on the denitrification gene expression, measured by quantitative reverse-transcription PCR (qRT-PCR) (Saleh-Lakha et al. 2009b).

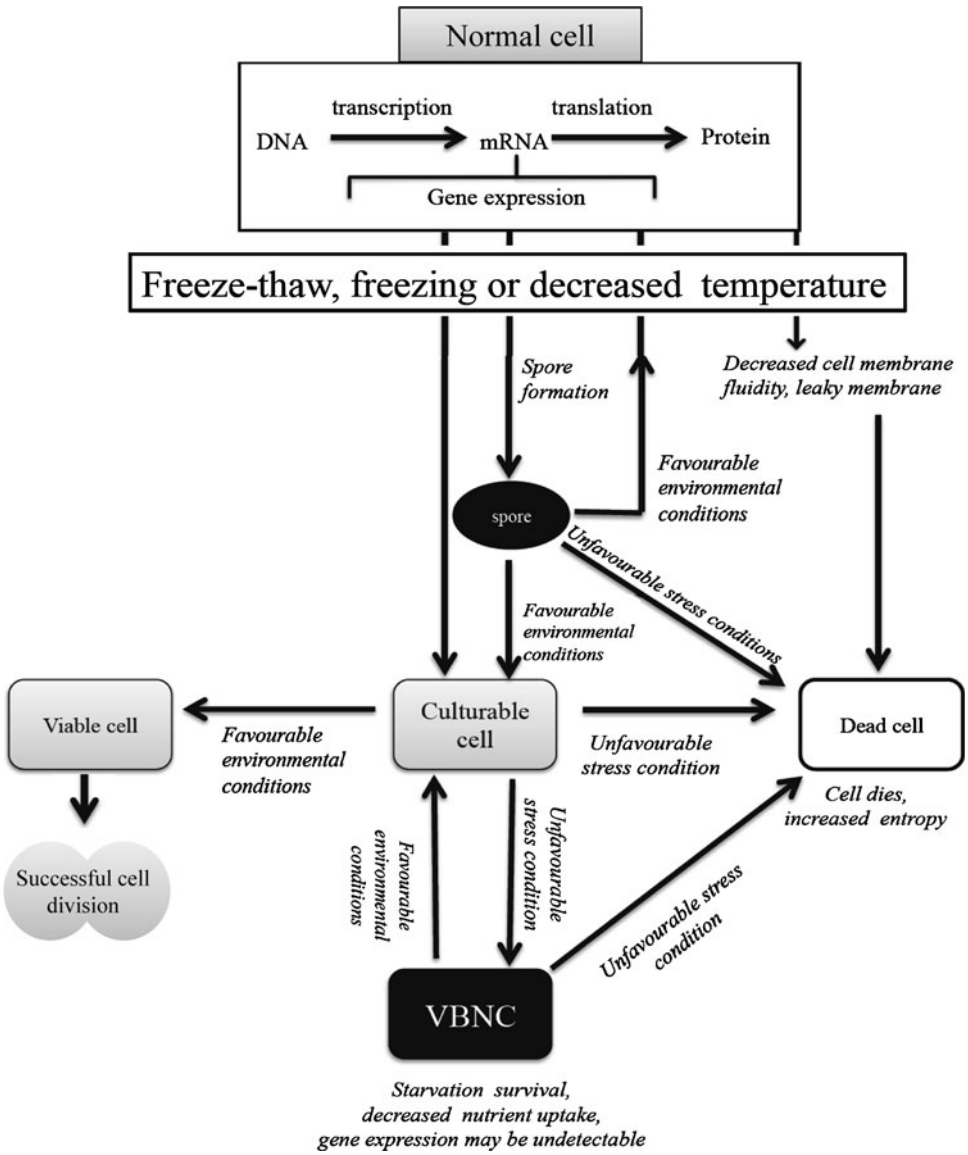

Fig. 1 Schematic representation of the effect of low temperatures on the occurrence of viable but nonculturable cells in Gram-negative bacteria and the occurrence of spore formation in Gram-positive cells

Denitrification activity was also measured by the accumulation of N_2O in the headspace in the presence of acetylene as the inhibitor of nitrous oxide reductase activity. *P. mandelii* cells grown at 20 and 30°C had about a 9-fold and 94-fold increase in *cnorB* (nitric oxide reductase) expression between 0 and 2 h, respectively. The average increase in *nirS* (nitrite reductase) gene expression was about 17-fold. Induction of *cnorB* and *nirS* gene expression for *P. mandelii* cells grown at 10°C did not occur in the first 4 h. Cumulative denitrification at 10 h was 6.6 µmol for *P. mandelii* cells grown at 10 and 20°C, and 30 µmol for cells grown at 30°C.

A pioneering study by Tsai and Olson (1990) on the effect of both temperature and mercury on the expression of mercury resistance genes revealed some interesting knowledge. Twenty *Pseudomonas* isolates were grown in the presence of 25 µg Hg^{2+} or 3 µg CH_3-Hg^{+} per ml, after which the total cellular RNA extracted and the *merA* (mercuric reductase) and *merB* (organomercurial lyase) transcripts were detected and quantified by Northern blot RNA hybridization. At 4°C and growth in the presence of 25 µg/ml Hg^{2+}, *merA* expression in mid-exponential growth for six isolates increased. *merA* transcript production by 18 of the 20 isolates increased when they were grown in the presence of 15 µg of Hg^{2+}/ml. Only 8 isolates had increased *merB* transcripts. In one *Pseudomonas* isolate (OR2), *merA* transcripts increased in a linear manner for 8 h at 37°C and for 4 h at 23°C, in the presence of Hg^{2+} (Tsai and Olson 1990), demonstrating the marked effect of temperature.

Flavobacterium psychrophilium is the causative agent of bacterial coldwater disease (BCWD) and rainbow trout fry syndrome (RTFS) as well as some other fish species (Hesami et al. 2011). Cold regulated genes were identified with a virulent strain of this bacterium. The researchers

discovered that at 8 versus 20°C, the following predicted genes, i.e., two component histidine kinase, ATP-dependent RNA helicase, multidrug ABC transporter permease, an outer membrane (OM) antigen OMA87, an M43 cytophagalysin zinc-dependent metalloprotease, a hypothetical protein and four housekeeping genes were up-regulated. However, the 16S ribosomal RNA copy number was the same at both temperatures (Hesami et al. 2011). The methods used were suppressive subtraction hybridization (SSH) and reverse-transcription quantitative real-time PCR.

Cold temperatures induce the VBNC state in cells of *Ralstonia solanacearum* biovar 2, the tropical agent that causes bacterial wilt disease (brown rot) in potato (van Elsas et al. 2000, 2007). A temperature of 4°C was observed to induce a VBNC state in *R. solanacearum* biovar 2, whereas at 20°C this induction was essentially absent (Van Overbeek et al. 2004). In another study conducted with *R. solanacearum* cultures, it was shown that copper ions ($CuSO_4$ at concentrations of 5 μM and higher) also induced the VBNC state (Grey and Steck 2001). The cellular mechanisms in the induction of the VBNC state in *R. solanacearum* can be different when exposed to copper or to low temperatures. However, in both cases VBNC cultures were resuscitated to culturable state following injection into the tomato plants, which is a suitable host for testing virulence. This illustrates that *R. solanacearum* VBNC cells can revert to culturable state when exposed to their natural environment (Grey and Steck 2001; Van Overbeek et al. 2004). Also, culturable *R. solanacearum* biovar 2, resulting from conversion from the VBNC state, had lost their ability to cause wilting disease in tomato plants (Van Overbeek et al. 2004), and therefore it was concluded that virulence was lost upon cold-induced stress in *R. solanacearum*.

Vibrio cholera O1 cells exposed to 5°C also rapidly lost culturability and a change to a distinct coccoid morphology was noticed upon outgrowth of residual culturable cells (Carroll et al. 2001). Concomitant with these occurrences was the induction of the cold-shock protein CS7.2 as demonstrated by Western blot assays, and a decrease in the cellular level of cholera toxin. Exposure of cultures to a cold temperature led to a cascade of cellular and morphological effects in *V. cholera*, and this may also be the case in *R. solanacearum*. It is plausible that exposure of thermophilic and mesophilic Gram-negative bacterial species to cold temperatures can lead to de novo production of cold-inducible proteins, altered metabolism and possibly to irreversible damage of the cytoplasm and even DNA, compared with the oxidative-stress-induced VBNC state in bacteria (Desnues et al. 2003) (Fig. 1).

Experimental designs to demonstrate phenotypic deviations in the VBNC state from culturable cells is complex. VBNC cells must be separated from their culturable and dead counterparts present in the same population. Even in liquid pure cultures this is a task. Calculation of nonculturable cell numbers in populations can be done by total cell numbers, determined by direct microscopic cell counts and substracting colony numbers on agar plates. Calculation of VBNC cells in a nonculturable population can be done by viability straining. Enrichment for living nonculturable cell fractions is commonly done by a combination of staining cells with green SYTO fluorescent dye and flow cytometry (Gaforio et al. 2002). SYTO green dye stains nucleic acids (DNA/RNA) in cells with intact membranes, whereas propidium iodide (PI), used as a counter dye, stains cells with compromised membranes ('dead' cells). Cells with intact membranes transport the green SYTO dye across the membrane and these cells emit a green fluorescence upon exposure to UV. Occurrence of green cells thus indicate the presence of the 'living' nonculturable cells and these cells must be separated from 'dead' red-stained cells in order to enrich for VBNCs in the culture suspension.

Separation of living and dead nonculturable cells in cold-exposed suspensions of *R. solanacearum* (bv 2) by flow cytometry was described by Van Overbeek et al. (2004) (Fig. 2). Two distinct cell fractions were distinguishable upon SYTO9/PI staining of cold-induced nonculturable cells followed by flow cytometer separation, whereas following the same procedure with cells exposed to 20°C, only one fraction was present. A third intermediate fraction could be discriminated by flow cytometry in cold-induced nonculturable *R. solanacearum* cells. These cells stained with both dyes at an equal level and because of the uncertainty of their metabolic and cellular status, these cells were not used for further experimentation by Van Overbeek et al. (2004). Flow cytometry sorting of green-stained nonculturable cells thus resulted in an enriched fraction of VBNCs; however, the occurrence of low-level contamination with culturable cells cannot be completely excluded (Fig. 2).

To demonstrate that *R. solanacearum* (biovar 2) growth in test plants occurred from a conversion of VBNC to culturable forms and not from outgrowth of residual culturable cells, it was necessary to treat plants with serially diluted samples of the collected VBNC suspension as in the study done on *V. vulnificus* (Whitesides and Oliver 1997). Plants treated with highest cell dilutions: (1) were infected with the introduced cells and these cells were recoverable on agar medium; (2) were shown to be avirulent; and (3) multiplied in test plants for many generations as was calculated on the basis of estimated retrieved versus introduced cell numbers (Van Overbeek et al. 2004). This led to the conclusion that resuscitation and outgrowth of *R. solanacearum* (biovar 2) VBNCs are likely to occur in host plants suitable for this pathogen, although these events can

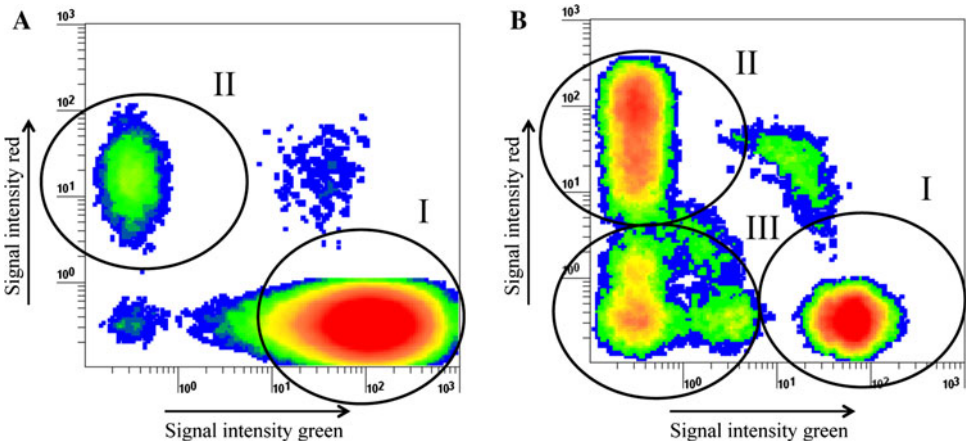

Fig. 2 Flow cytometry separation of 'living' (*green* SYTO 9-stained) and 'dead'(*red* PI-counterstained) *Ralstonia solanacearum* biovar 2 cells exposed to 20°C (**a**) (control treatment) and 4°C (**b**) (VBNC-inducing treatment). *Green* (*I*), *red* (*II*) and intermediate (*III*) stained cells from the cold-treated cell suspension was separated into distinct fractions and the fraction containing *green* cells were plated (for testing of absence of colony growth), serially diluted and injected into test plants as described in the text. The axes are the fluorescent intensities in the selected flow cytometer channels at the correct wavelengths

be disputed because the resuscitated cells had irreversibly lost their virulence.

Another approach to study bacterial genes at suboptimal temperatures is to express genes from psychrophilic/psychrotrophic bacteria in mesophilic bacterial hosts, which results in the host cells becoming heat-sensitive but capable of growth at lower temperatures (de Lorenzo 2010). These cells are then useful in heterologous gene expression.

In general, almost all major cellular components seem to respond to cold adaptation. The most studied biological genetic sensors known to take part in the cold adaptation include: (1) *csp* genes coding for Csps or cold-shock proteins that are expressed immediately after downshifts in temperature; (2) *cap* genes coding for Caps (cold acclimation proteins) that are expressed during prolonged growth at low temperatures (acclimation); (3) *afpA* genes coding for the antifreeze proteins or AFPs that are expressed to avoid freezing at subzero temperatures; and (4) genes for IBPs (ice-binding proteins) whose exact function is still unknown, but may act as recrystallization inhibitors to protect membranes in the frozen state.

Cold-shock proteins (Csps) and cold adaptive proteins (Caps)

Besides *E. coli*, *cspA* gene regulation is often researched in a number of Gram-negative bacteria belonging to other mesophilic bacteria, and bacteria from cold environments (Bej et al. 2000; Bryan et al. 1999; Carroll et al. 2001; Francis and Stewart 1997; Goldstein et al. 1990; Horton et al. 2000; Jeffreys et al. 1998; Mojib et al. 2008, 2011; Bej and Mojib 2009; Panicker et al. 2002, 2010). The CspA family of proteins in *E. coli* consists of nine homologs (Phadtare et al. 1999) that function as RNA chaperones by minimizing secondary structures on mRNAs. As a result, efficient translation at suboptimal temperatures or function as transcription regulators and transcription antiterminators (Bae et al. 2000). *E. coli* CspA, CspB, CspG and CspI are cold inducible, whereas CspC and CspE are constitutively expressed and have been shown to function as suppressors of the chromosome partitioning during cell division in *E. coli* (Yamanaka et al. 1994). The most studied cold shock family of proteins is the *cspA*, the promoter of which is constitutively expressed at 37°C, though its activity increases following cold shock (at 10°C) (Fang et al. 1997). Therefore, the regulation of *cspA* at cold temperatures occurs at the posttranscriptional level by stabilizing the *cspA* transcript. The expression of a homolog of *E. coli cspA* was detected in the psychrotolerant Gram-positive *Arthrobacter protophormiae* and Gram-negative *Pseudomonas fluorescens* at 4 and 22°C by northern blot hybridization, with elevated expression at colder temperatures (Ray et al. 1994). The expression of *E. coli cspF* and *cspH* has not been associated with any particular growth condition or phenotype (Giaquinto et al. 2007). Non cold-inducible *E. coli* CspD functions as a DNA replication inhibitor during the stationary growth phase and are induced upon glucose starvation at 37°C (Yamanaka and Inouye 1997); protection against UV damage of DNA in *Halobacterium* NRC-1 and in *Janthinobacterium* Ant5-2 (Baliga et al. 2004; Mojib et al. 2011); and formation of biofilm and motility leading to the formation of the persister population (Kim et al. 2010; Kim and Wood 2010). Expression of 13 common CSPs was detected upon temperature downshift in Arctic *Rhizobium* spp. and these Arctic rhizobia expressed relatively elevated Csps under freezing condition (−10°C) (Cloutier et al. 1992). The expression of 14 Csps was reported in the Arctic

psychrophile *Aquaspirillum arcticum* using O'Farell 2-D protein gel electrophoresis (Roberts and Inniss 1992). A family of dimeric major cold-shock protein homologs was identified, confirmed by immunoblotting, and purified from the psychrotolerant *B. cereus* WSBC 10201 (Mayr et al. 1996). Cold-shock proteins were also identified in *Psychrobacter arcticus* from a 20,000–40,000 year old Siberian permafrost core sample (Zheng et al. 2006).

Only four genes encoding the Caps have been sequenced and characterized: *capA* from *Arthrobacter globiformis* (Berger et al. 1996, 1997); *capB* from *Pseudomonas* strain 30-3 (Panicker et al. 2010) and *capA* and *capB* from *Pseudomonas fragi* (Michel et al. 1997). These genes share 60–70% sequence identity with CspA amino acid residues and possess the conserved RNP-1 and RNP-2 ssDNA or RNA binding motifs (Fig. 3). However, lack of sufficient homologies of the 5′-UTR sequence of *Pseudomonas* 30-3 *capB* with *cspA* family of genes suggests that although the CspA and CapB proteins retained the same function, the trigger for *capB* expression at low temperature is different from that of *cspA* (Thieringer et al. 1998). The expression of CspA in mesophilic bacteria including *E. coli* has been reported to be transient (Thieringer et al. 1998), whereas in bacteria inhabiting perennially cold environments including the Antarctic and Arctic environments the sustained expression of Caps with the conserved RNA destabilization function seems essential for the expression of essential proteins, thereby maintaining active growth and metabolism (Panicker et al. 2010).

Antifreeze proteins (AFP) and ice-binding proteins (IBP)

Although the production of AFPs is one of the most important survival strategies in cold-tolerant eukaryotes, especially Antarctic fishes, many bacteria also express proteins that function as AFP (Berry et al. 2005; Duman and Olsen 1993; Gilbert et al. 2004, 2005; Kawahara et al. 2004; Muryoi et al. 2004; Yamashita et al. 2002). Antifreeze proteins (AFPs) protect against freezing damage by depressing the freezing point of water in a noncolligative manner either by preventing ice nucleation or inhibit water molecules to form a larger ice crystal by attaching to tiny ice crystals, which, once formed, may be detrimental to the organisms during freezing conditions. Recently, an ice-binding protein (IBP) has been described in the sea-ice bacterium *Colwellia* sp. SLW05 18 (Raymond et al. 2007) and an antifreeze lipoprotein from *Moraxella* sp. of Antarctic origin (Yamashita et al. 2002). The IBP binds to ice crystal lattices and prevents them from recrystallization, thereby protecting the bacterium from freeze-damage of the cytoplasmic membrane. The IBP has been found in sea-ice diatom and snow molds, but the amino acid sequences seem to be diverse (Bej and Mojib 2009). Different structural motifs found in AFPs isolated and characterized from a wide range of organisms or even among bacterial species is just one of the paradoxical elements in the study of AFPs (Sharp 2011). The comparison of the *Pseudomonas putida* AfpA amino acid residues with other bacteria exhibited a range from the lowest 23.4% (*Pseudomonas ingrahamii*) to the highest 32.7% (*Polaribacter irgensii*) identity (Bej and Mojib 2009).

The latest and most detailed study of the mechanism of action of AFP to distinguish between different structural states of water has been elucidated by solving the first crystal structure of an Antarctic bacterium *Marinomonas primoryensis* (Garnham et al. 2011). *M. primoryensis* produces an exceptionally large (1.5 MDa) Ca^{2+}-dependent AFP (*Mp*AFP). By showing the extensive X-ray crystallographic picture of the AFP–ice interaction, Garnham et al. (2011) elucidated the "anchored clathrate" mode of binding of the 34-kDa Ca^{2+}-bound parallel beta-helix domain of *Mp*AFP in which an array of ice-like surface water is anchored via hydrogen bonds directly to the polypeptide backbone and adjacent side chains. Many other long-standing questions about the AFP mechanism of action and speculations about the types of interactions that mediate ice binding are discussed in a recent review (see

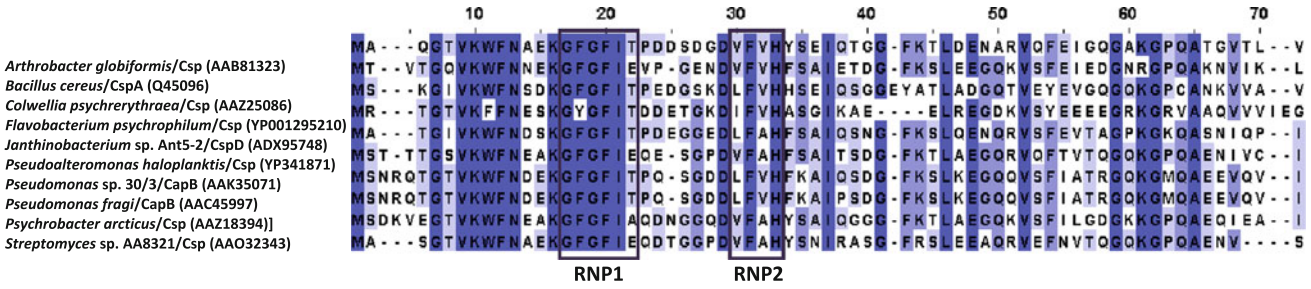

Fig. 3 Multiple sequence alignment of cold-shock proteins (*Csps*) and cold acclimation protein (*Caps*) identified in microorganisms from cold environments using T-coffee software (http://www.ebi.ac.uk/Tools/msa/). The accession numbers for respective sequences from NCBI are shown in *parentheses*. The RNP1/RNP2 motifs shown in *square boxes* are conserved domains in Csps and Caps isolated from different bacterial species

review by Sharp 2011). A remarkable diversity of AFP structures with common function of ice binding could partly be due to their independent evolutionary origins (Cheng 1998) and partly to the surface heterogeneity of their natural ligand, ice (Davies et al. 2002). An increasing number of Antarctic bacterial species have been shown to express IBP with possible antifreeze function. Therefore, the role of IBPs and AFPs in Antarctic bacterial isolates seems crucial for their survival in such a harsh environment (Raymond et al. 2007).

Other aspects of bacterial gene expression research

Other scientific areas that require gene expression research will be in the use of bacteria/microorganisms as biocontrol agents in the suppression of root diseases in plants, bacterial gene expression in the rhizosphere and the differential responses of microorganisms to elevated atmospheric carbon dioxide and warming temperatures in soil, sediment and water. This knowledge will be central to the understanding of the effects of both short-term and long-term temperature increases of microbial-mediated biogeochemical processes in diverse geographical locations on the planet. For example, climate models predict that by 2080 the summer temperatures in the Arctic regions will have increased by 4–7.5°C (Anisimovo and Fitzharris 2001). It has also been reported that the annual temperatures on the Antarctic Peninsula have risen about 2.8°C during the last 50 years making it the most rapidly warming region in the Southern Hemisphere (Turner et al. 2005; Vaughan et al. 2001, 2003). Microbial gene expression under these changing conditions, different from present-day conditions, will need to be understood so that greenhouse gases such as carbon dioxide, nitrous oxide and methane in warming locations can be better managed.

Summary

Since the report that an *E. coli* culture expressed a cold shock gene, i.e., *cspA*-encoded major cold-shock protein, CS7.4, (Goldstein et al. 1990), subsequent investigations revealed that a suite of genes responds to changing environment optimal to suboptimal growth temperatures. During the last two decades an intriguing question has been challenging the scientific community about the identity of the cold-responsive key central genetic regulator necessary for bacterial survival and adaptation when mesophilic as well as the psychrophilic and psychrotolerant bacteria encounter suboptimal growth temperatures. A recent review by Shivaji and Prakash (2010) listed the possible cellular sensors, physiological parameters and genetic regulators thus far reported for bacteria to survive and adapt in cold environments. However, the central genetic regulator for sensing changes in temperatures still remain elusive. To better understand the whole cell physiology, such as in a gene expression microarray analysis, or expression of one or more genes using PCR analysis under suboptimal conditions such as low temperatures, additional research is needed. Decreased temperature is one of the important environmental conditions under which some microorganisms are capable of persistence, growth, reproduction and interactions. Gene expression analysis will allow researchers to better understand the behavior of pathogens in the environment, in situ toxin production, gene transfer (transformation, conjugation, transduction), antibiotic resistances, metal resistances, biocontrol mechanisms, degradation of toxic pollutants and biogeochemical cycling. Global warming may result in both non-pathogenic and pathogenic bacteria moving into new environmental niches where new problems, with plant and animal pathogens and waterborne diseases, may arise. Gene expression studies will provide a better understanding of the interactions between bacteria, environment and their hosts. This research will be especially important where bacteria and other microorganisms display differential responses to temperature fluctuations. Moreover, it will deliver new and relevant gene expression data that were not possible a decade ago. Another wave of bacterial biotechnology applications will be possible based on gene expression and/or gene expression modules where bacterial cells are engineered to perform specific tasks in an efficient and cost-effective manner for new applied, industrial and environmental applications. Relevant low-temperature gene expression capabilities in the hosts may allow engineered microorganisms to be placed in environmental niches such as sewage treatment, mining and toxic waste sites for safe and specific applications.

Acknowledgments Research by J.T.T. was supported by the NSERC (Canada) Discovery program. The logistic support for research on Antarctic bacteria by A. Bej was supported by the Tawani Foundation and Col (Ret) Jim Pritzker; Marty Kress of VCSI; NASA's Exobiology program; Richard Hoover of NASA; Rasik Ravindra of NCAOR; Ashit Swain of GSI and Indian Maitri Station in Antarctica.

References

Anisimovo O, Fitzharris B (2001) Polar regions (Arctic and Antarctic). In: McCarthy JJ, Canziani OF, Leary NA, Dokken DJ, White KS (eds) Climate change 2001: impacts, adaptation and vulnerability. Cambridge University Press, Cambridge, pp 801–841

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

Baliga NS, Bjork SJ, Bonneau R, Pan M, Iloanusi C, Kottemann MCH, Hood L, DiRuggiero J (2004) Systems level insights into the stress response to UV radiation in the halophilic archaeon *Halobacterium NRC-1*. Genome Res 14(6):1025–1035

Bej AK, Mojib N (2009) Cold adaptation in Antarctic biodegradative microorganisms. In: Bej AK, Aislabie JM, Atlas RM (eds) Polar microbiology: the ecology, biodiversity and bioremediation potential of microorganisms in extremely cold environments. CRC Press, Boca Raton, pp 157–177

Bej A, Saul D, Aislabie J (2000) Cold tolerance of alkane-degrading bacteria isolated from soil near Scott Base, Antarctica. 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

Bergman NH, Passalacqua KD, Hanna PC, Qin ZS (2007) Operon prediction for sequenced bacterial genomes without experimental information. Appl Environ Microbiol 73:846–854

Berry MJ, Griffiths A, Hill PJ, Laybourne-Parry J, Mills SV (2005) Processes and organisms for the production of anti-freeze proteins. United States Patent 6:887, 984B2

Bryan PJ, Steffan RJ, Foster JW, Bej AK (1999) Adaptive response to cold temperatures in *Vibrio vulnificus*. Curr Microbiol 38:168–175

Carroll JW, Mateescu MC, Chava K, Colwell RR, Bej AK (2001) Response and tolerance of toxigenic *Vibro cholerae* O1 to cold temperatures. Antonie Van Leeuwenhoek 79:377–384

Cheng CH (1998) Evolution of the diverse antifreeze proteins. Curr Opin Genet Dev 8:715–720

Cloutier J, Prévost D, Nadeau P, Antoun H (1992) Heat and cold shock protein synthesis in arctic and temperate strains of rhizobia. Appl Environ Microbiol 58(9):2846–2853

Davies PL, Baardsnes J, Kuiper MJ, Walker VK (2002) Structure and function of antifreeze proteins. Philos Trans R Soc Lond B Biol Sci 357:927–935

de Lorenzo V (2010) Genes that move the window of viability of life: lessons from bacteria thriving at the cold extreme. Bioessays 33:38–42

Desnues B, Cuny C, Grégori G, Dukan S, Aguilaniu H, Nyström T (2003) Differential oxidative damage and expression of stress defence regulons in culturable and non-culturable *Escherichia coli* cells. EMBO Rep 4:400–404

Dubois JW, Hill S, England LS, Edge T, Masson L, Trevors JT, Brousseau R (2003) A DNA microarray-based assay for the characterization of commercially formulated microbial products. J. Microbiol. Meths. 58:251–262

Ducey TF, Vanotti MB, Shriner AD, Szogi AA, Ellison AQ (2010) Characterization of a microbial community capable of nitrification at cold temperature. Bioresour Technol 101:491–500

Duman JG, Olsen TM (1993) Thermal hysteresis protein activity in bacteria, fungi, and phylogenetically diverse plants. Cryobiology 30:322–328

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

Francis KP, Stewart GS (1997) Detection and speciation of bacteria through PCR using universal major cold-shock protein primer oligomers. J Ind Microbiol Biot 19:229–286

Gaforio JJ, Serrano MJ, Ortega E, Algarra I, Alvarez de Cienfuegos G (2002) Use of SYTOX green dye in the flow cytometric analysis of bacterial phagocytosis. Cytometry 48:93–96

Garnham CP, Campbell RL, Davies PL (2011) Anchored clathrate waters bind antifreeze proteins to ice. Proc Natl Acad Sci USA 108:7363–7736

Giaquinto L, Curmi PM, Siddiqui KS, Poljak A, DeLong E, DasSarma S, Cavicchioli R (2007) Structure and function of cold shock proteins in archaea. J Bacteriol 189:5738–5748

Gilbert JA, Hill PJ, Dodd CER, Parry JL (2004) Demonstration of antifreeze protein activity in Antarctic lake bacteria. Microbiology 150:171–180

Gilbert JA, Davies PL, Parry JL (2005) A hyperactive, Ca2+-dependent antifreeze protein in an Antarctic bacterium. FEMS Microbiol Lett 245(1):67–72

Goldstein J, Pollitt NS, Inouye M (1990) Major cold shock protein of *Escherichia coli*. Proc Natl Acad Sci USA 87(1):283–287

Grey BE, Steck TR (2001) The viable but noncultural state of *Ralstonia solanacearum* may be involved in long-term survival and plant infection. Appl Environ Microbiol 67:3866–3872

Hesami S, Metcalf DS, Lumsden JS, MacInnes JL (2011) Identification of cold temperature regulated genes in *Flavobacterium psychrophilium*. Appl Environ Microbiol. doi:10.1128/AEM.01717-10

Horton A, Hak K, Steffan RJ, Foster J, Bej A (2000) Adaptive response to cold temperatures and characterization of *cspA* in *Salmonella typhimurium* LT2. Anton Leeuw Int J G 77(1):13–20

Jeffreys AG, Hak KM, Steffan RJ, Foster JW, Bej AK (1998) Growth, survival and characterization of *cspA* in *Salmonella enteritidis*. Curr Microbiol 36:29–35

Kawahara H, Yoko N, Kazuhiro O, Muryoi N, Jiro N, Hitoshi O (2004) Production of two types of ice crystal-controlling proteins in Antarctic bacterium. J Biosci Bioeng 98(3):220–223

Kim Y, Wood TK (2010) Toxins Hha and CspD and small RNA regulator Hfq are involved in persister cell formation through MqsR in *Escherichia coli*. Biochem Biophys Res Commun 391:209–213

Kim Y, Wang X, Zhang XS, Grigoriu S, Page R, Peti W, Wood TK (2010) *Escherichia coli* toxin/antitoxin pair MqsR/MqsA regulate toxin CspD. Environ Microbiol 12:1105–1121

Mayr B, Kaplan T, Lechner S, Scherer S (1996) Identification and purification of a family of dimeric major cold Shock protein homologs from the psychrotrophic *Bacillus cereus* WSBC 10201. J Bacteriol 178:2916–2925

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

Mojib N, Hoover RB, Bej AK (2008) Diversity and cold adaptation of microorganisms isolated from the Schirmacher Oasis, Antarctica. Proc of SPIE 7097:70970K-1

Mojib N, Andersen DT, Bej AK (2011) Structure and function of a cold shock domain fold protein, CspD, in *Janthinobacterium* sp. Ant5-2 from East Antarctica. FEMS Microbiol Lett 319:106–114

Muryoi N, Sato M, Kaneko S, Kawahara H, Obata H, Yaish MW, Griffith M, Glick BR (2004) Cloning and expression of afpA, a gene encoding an antifreeze protein from the arctic plant growth-promoting rhizobacterium *Pseudomonas putida* GR12–2. J Bacteriol 186(17):5661–5671

Mykytczuk NCS, Trevors JT, Ferroni GD, Leduc LG, Foote SJ, Twine SM (2011) Proteomic insights into cold adaptation in psychrotrophic and mesophilic strains of *Acidithiobacillus ferrooxidans*. Anton van Leeuwen 100:259–277

Panicker G, Aislabie J, Saul D, Bej AK (2002) Cold tolerance of *Pseudomonas* sp. 30-3 isolated from oil-contaminated soil, Antarctica. Polar Biol 25:5–11

Panicker G, Mojib N, Nakatsuji T, Aislabie J, Bej AK (2010) Occurrence and distribution of *capB* in Antarctic microorganisms and study of its structure and regulation in the Antarctic

biodegradative *Pseudomonas* sp. 30/3. Extremophiles 14:171–183

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

Ray MK, Sitaramamma T, Ghandhi S, Shivaji S (1994) Occurrence and expression of cspA, a cold shock gene, in Antarctic psychrotrophic bacteria. FEMS Microbiol Lett 116(1):55–60

Raymond JA, Fritsen C, Shen K (2007) An ice-binding protein from an Antarctic sea ice bacterium. FEMS Microbiol Ecol 61:214–222

Roberts ME, Inniss WE (1992) The synthesis of cold shock proteins and cold acclimation proteins in the psychrophilic bacterium *Aquaspirillum arcticum*. Curr Microbiol 25(5):275–278

Rodrigues DF, Tiedje JM (2008) Coping with our cold planet. Appl Environ Microbiol 74:1677–1686

Saleh-Lakha S, Shannon KE, Henderson SL, Goyer C, Trevors JT, Zebarth BJ, Burton DL (2008) Nitric oxide reductase gene expression and nitrous oxide production in nitrate-grown *Pseudomonas mandelii*. Appl Environ Microbiol 74:6876–6879

Saleh-Lakha S, Shannon KE, Henderson SL, Goyer C, Trevors JT, Zebarth BJ, Burton DL (2009a) Effect of nitrate and acetylene on *nirS*, *cnorB* and *nosZ* expression and denitrification activity in *Pseudomonas mandelii*. Appl Environ Microbiol 75:5082–5087

Saleh-Lakha S, Shannon KE, Henderson SL, Goyer C, Trevors JT, Zebarth BJ, Burton DL (2009b) Effect of pH and temperature on denitrification genes expression and activity in *Pseudomonas mandelii*. Appl Environ Microbiol 75:3903–3911

Saleh-Lakha S, Shannon K, Goyer C, Trevors JT (2011) Challenges in quantifying microbial gene expression in soil using quantitative reverse transcription real-time PCR. J Microbiol Methods 85:239–243

Sharp KA (2011) A peek at ice binding by antifreeze proteins. Proc Natl Acad Sci USA 108(18):7281–7282

Shivaji S, Prakash JS (2010) How do bacteria sense and respond to low temperature? Arch Microbiol 192(2):85–95

Thieringer H, Jones P, Inouye M (1998) Cold shock and adaptation. BioEssays 20:49–57

Tsai YL, Olson BH (1990) Effects of Hg^{2+}, CH_3-Hg^{+} and temperature on the expression of mercury resistance genes in environmental bacteria. Appl Environ Microbiol 56:3266–3272

Turner J, Colwell SR, Marshall GJ, Lachlan-Cope TA, Carleton AM, Jones PD, Lagun V, Reid PA, Iagovkina S (2005) Antarctic climate change during the last 50 years. Int J Climatol 25:279–294

Van Elsas JD, Kastelein P, van Bekkum P, van der Wolf JM, de Vries PM, van Overbeek LS (2000) Survival of *Ralstonia solanacearum* biovar 2, the causative agent of potato brown rot, in field and microcosm soils in temperate climates. Phytopathology 90:1358–1366

Van Elsas JD, Jansson JK, Trevors JT (2007) Modern soil microbiology II. CRC Press–Taylor and Francis, Boca Raton, p 646

Van Overbeek LS, Bergervoet JHW, Jacobs F, van Elsas JD (2004) The low-temperature-induced viable-but-nonculturable state affects the virulence of *Ralstonia solanacearum* biovar 2. Phytopathology 94:463–469

Vaughan DG, Marshall GJ, Connolley WM, King JC, Mulvaney R (2001) Climate change: devil in the detail. Science 293:1777–1779

Vaughan DG, Marshall GJ, Connolley WM, Parkinson C, Mulvaney R, Hodgson DA, King JC, Pudsey CJ, Turner J (2003) Recent rapid regional climate warming on the Antarctic Peninsula. Clim Change 60:243–274

Whitesides MD, Oliver JD (1997) Resuscitation of *Vibrio vulnificus* from the viable but nonculturable state. Appl Environ Microbiol 63:1002–1005

Yamanaka K, Inouye M (1997) Growth-phase-dependent expression of *cspD*, encoding a member of the CspA family in *Escherichia coli*. J Bacteriol 179:5126–5130

Yamanaka K, Mitani T, Ogura T, Niki H, Hiraga S (1994) Cloning, sequencing, and characterization of multicopy suppressors of a mukB mutation in *Escherichia coli*. Mol Microbiol 13:301–312

Yamashita Y, Nakamura N, Omiya K, Nishikawa J, Kawahara H, Obata H (2002) Identification of an antifreeze lipoprotein from *Moraxella* sp. of Antarctic origin. Biosci Biotechnol Biochem 66(2):239–247

Zheng S, Ponder MA, Shih JYJ, Tiedje JM, Thomashow MF, Lubman DM (2006) A proteomic analysis of *Psychrobacter articus* 273-4 adaptation to low temperature and salinity using a 2-D liquid mapping approach. Electrophoresis 28(3):467–488