Review

Mechanism of bacterial adaptation to low temperature

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Survival of bacteria at low temperatures provokes scientific interest because of several reasons. Investigations in this area promise insight into one of the mysteries of life science – namely, how the machinery of life operates at extreme environments. Knowledge obtained from these studies is likely to be useful in controlling pathogenic bacteria, which survive and thrive in cold-stored food materials. The outcome of these studies may also help us to explore the possibilities of existence of life in distant frozen planets and their satellites.

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1. Introduction

During the last two decades, a number of investigations have been performed at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad involving some Antarctic bacterial strains and also in some other laboratories on the biochemical and genetic basis of bacterial cold tolerance. This report summarizes the previous findings and highlights some new aspects. The differentiation between psychrotrophs and psychrophiles is, by and large, ignored now-a-days and all the cold-tolerant bacteria are called psychrophiles.

Investigations conducted earlier on the mechanism of cold adaptation were mostly focussed on isolation and characterization of cold-active and/or thermolabile enzymes and comparison of their amino acid composition to that of their counterparts, obtained from mesophilic organisms. This article reveals the limitations of such approach, as evidenced by a recent report. It also deals with recent advances in the understanding of the maintenance of membrane fluidity and metabolism at low temperature. The ability of cold-tolerant bacteria to continue growth and metabolism at subzero temperatures is also emphasized on the basis of evidences obtained from literature. The role of various stress proteins, antifreeze proteins and low molecular weight compounds is discussed. Possible involvement of viable but nonculturable (VBNC) cells and regulatory role of the cellular machinery for the degradation of RNA have been highlighted. The interlinked nature of bacterial adaptations to various stress conditions is mentioned and prospective future studies are suggested.

2. Sensing of environmental temperature

Phosphorylation and dephosphorylation of a membrane protein of an Antarctic bacterium *Pseudomonas syringae* in response to upshift and downshift of temperature, both *in vitro* and *in vivo*, suggested a possible role of the membrane proteins in sensing environmental temperature (Ray *et al* 1994a). The phosphorylated membrane protein induced phosphorylation of a 66 kDa cytosolic protein at the tyrosine residues by a novel protein tyrosine kinase (Jagtap and Ray 1999), the role of which in the signal transduction process is yet to be established. Differential phosphorylation of lipopolysachharides at high and low temperature of

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Abbreviations used: AFPs, antifreeze proteins; CSPs, cold-shock proteins; HSPs, heat shock proteins; TH, thermal hysteresis; VBNC, viable but nonculturable.

the same organism was also demonstrated (Ray *et al* 1994b). It might also have a role in the sensing process. Several lines of investigations, reported earlier, implied the involvement of ribosomes in sensing a change in the environmental temperature (Ray *et al* 1998 and references therein). Increase in the *lhkA* mRNA level during growth of *Listeria monocytogenes* at low temperature was reported some time back (Liu *et al* 2002). The histidine kinase, Hik 33, is known to sense low temperature in the cyanobacterium *Synechocystis* sp. strain PCC 6803 (Suzuki *et al* 2001).

3. Structural adaptation of enzymes

Enzymes catalyze hundreds of chemical reactions in the cell, crucial for the maintenance of life. Hence adaptation of the cell to the low temperature calls for presence of intracellular enzymes which are active at low temperature. Isolation and characterization of DNA-dependent RNA polymerase (Uma *et al* 1999), ribonuclease (Reddy *et al* 1994) and alkaline phosphatase (Chattopadhyay *et al* 1995) from different Antarctic strains were reported earlier. Other cold-active enzymes, recently obtained from different cold-tolerant bacteria, include protein-tyrosine phosphatase (Tsuruta *et al* 2004), α-amylase and β-galactosidase (Groudieva *et al* 2004) and aminopeptidase (Huston *et al* 2004).

Generally speaking, thermal stability of enzymes is associated with structural rigidity, which restricts the interaction between enzyme and its substrate. Hence thermophilic enzymes are poor catalysts at room temperature. On the contrary, cold adaptability requires structural flexibility, which favours greater complementarity at low energy cost. It provides a rational explanation of the high specific activity of some cold-adapted enzymes (Gerday *et al* 2000).

Attempts have been made from time-to-time to isolate a cold-active enzyme from a cold-tolerant bacterium and to isolate from a mesophilic organism, the same enzyme having low activity or no activity at low temperature. Comparison of the amino acid composition of two enzymes has provided some clues to the structural basis of cold-tolerance. The criteria for cold-tolerance of the enzymes, as evident from this type of comparisons, include fewer residues of prolines or arginines, a lower ratio of arginine to arginine plus lysine, a decrease in hydrophobic residues coupled with an increase in polar residues and a decrease in the number of disulfide bonds (D'Amico *et al* 2002).

A recent investigation, led by James A Coker from the Department of Biochemistry and Molecular Biology of the Pennsylvania State University (USA), calls the validity of the above mentioned criteria in question. They isolated and characterized a psychrotolerant strain of *Arthrobacter* from the Antarctic dry-valley soil. A cold active β-galactosidase obtained from it was found to have temperature optimum near 18°C and it retained 50% activity at 0°C. It was 2.1 and 5.0 times more active than a β-galactosidase from *Escherichia coli* at 20°C and 10°C respectively. Earlier, another β-galactosidase was characterized from a cold-tolerant bacterium, viz. *Arthrobacter psychrolactophilus*. The enzyme had a temperature optimum around 40°C. Comparison between these two *Arthrobacter* enzymes revealed that except the decrease in the number of proline residues, most of the criteria for structural features, believed so far to confer cold-stability and cold-active nature of the enzyme, were not satisfied. Again, most of the trends suggested for cold-active enzymes were not found to be followed when the amino acid composition of the cold-active β-galactosidase was compared to that of a β-galactosidase isolated from *E. coli*. The thermolability of the enzyme was explained by the fact that it was a tetramer, which dissociated at 25°C into the inactive monomers (Coker *et al* 2003).

A previous investigation, conducted in the same laboratory on structural features contributing to thermostability of glcosyl hydrolases, revealed that distribution of hydrogen bonds, ion pairs and amino acid compositions did not differ significantly between the mesophilic and thermophilic proteins. Pairwise comparisons of thermophilic structures to mesophilic structures did not appear to lead to structural basis of thermostability (Panasik *et al* 2000). Thus it is evident that amino acid composition and gross structural comparisons do not always help in generalizing the structural basis of adaptation of enzymes activities at different extremities of temperature. Accumulating evidences in literature suggest that subtle, synergistic and co-operative intramolecular interactions (the nature of which remains unknown by and large) are likely to provide some explanations of thermostability or cold-active nature of enzymes (Wintrode *et al* 2000; Zartler *et al* 2001).

4. Maintenance of membrane fluidity

4.1 *Role of fatty acids*

With the decrease in environmental temperature, a number of changes are known to occur in the fatty acid profile of bacterial cell membrane to maintain an optimum fluidity. Conversion of saturated fatty acids into unsaturated fatty acids by the enzymes desaturases, induced at low temperature and preferential synthesis of short-chain fatty acids, branched chain fatty acids and anteiso-fatty acids to long-chain fatty acids, straight-chain fatty acids and iso fatty acids respectively are well-documented in the literature (Suutari and Laakso 1994). Enhanced synthesis of unsaturated fatty acids at lower temperature in one Gram-positive strain, viz. *Micrococcus roseus* and one Gram-negative strain, viz. *Sphingobacterium antarcticus* (Chattopadhyay and Jagannadham 2001 and references therein), both isolated

from Antarctic soil, was also observed. The essential role of desaturases in the growth of an Antarctic cyanobacterial strain at low temperature has been recently demonstrated (Chintalapati *et al* 2004). Conversion of *cis* fatty acids into *trans* fatty acids, by the enzyme *cis-trans* isomerase, is believed to facilitate the survival of bacteria at higher temperature, since the *trans* fatty acids are known to contribute to the decrease in membrane fluidity. In an Antarctic strain of *P. syringae*, decrease in membrane fluidity with concomitant increase in the amount of saturated and *trans* monounsaturated fatty acids was evidenced. However, the *cti* gene was found to be constitutively expressed in the same organism irrespective of growth temperature implying that production of *cis-trans* isomerase in this organism was post-transcriptionally regulated (Kiran *et al* 2005). The importance of one anteiso saturated fatty acid (a-$C_{15:0}$) in the cold tolerance of the food-borne pathogen *Listeria monocytogenes* was demonstrated earlier with the help of two cold-sensitive mutants (Annous *et al* 1997). Increase in the amount of hydroxy fatty acids in the lipopolysaccharides with concomitant increase in the fluidity (measured *in vitro*) of its hydrophobic phase in an Antarctic strain of *P. syringae*, incubated at low temperature, was also reported implicating an essential role of the hydroxy fatty acids in homeoviscous adaptation of outer membrane fluidity (Kumar *et al* 2002). Following a downshift of temperature from 37°C to 18°C, several genes were shown to be transcriptionally upregulated in a strain of *Bacillus subtilis*, using DNA microarray analysis. Among them were the genes, which encode enzymes involved in the degradation of the branched-chain amino acids (Kaan *et al* 2002). It is noteworthy that the intermediates of isoleucine and valine degradation (α-methylbutyryl-CoA and isobutyryl-CoA respectively) are utilized by the cellular machinery for the synthesis of branched chain fatty acids. Hence it is obvious that at low temperature, cells acquire fatty acids, required for the maintenance of membrane fluidity, not only through anabolic pathways but also by making use of catabolism.

4.2 *Role of carotenoids*

Several polar and non-polar carotenoids, synthesized in the Antarctic strains *Micrococcus roseus* and *Sphingobacterium antarcticus* were isolated and characterized. During fractionation of the subcellular components of these Antarctic bacteria, carotenoids were always found to be associated with the membranes and hence a role of the carotenoids in the regulation of membrane fluidity was postulated. The major carotenoid pigments from each organism were found to bind vesicles, made of both synthetic and natural lipids, and to rigidify them. A trend of increase in the amount of polar carotenoids and decrease in the amount of non-polar carotenoid was also observed in both the organisms when they were grown at low temperature, compared to their production profile obtained by growth at room temperature (Chattopadhyay and Jagannadham 2001 and references therein). A polar carotenoid was reported to decrease the membrane fluidity in a separate investigation (Subczynski *et al* 1992). Hence it was suggested that in response to the increase in the synthesis of membrane-fluidizing fatty acids, synthesis of membrane-rigidifying polar carotenoids was also enhanced to counterbalance the effects of fatty acids in the Antarctic bacteria (Chattopadhyay and Jagannadham 2001).

5. Metabolism at low temperature

The notion that bacteria survive at subzero temperatures in a state of suspended animation without dividing has been breached during the past few years following the demonstration of metabolic activity and growth of some permafrost bacteria below 0°C. On the basis of incorporation of ^{14}C-labelled acetate into lipids, growth characteristics of native bacterial population, obtained from Siberian permafrost, were studied at various temperatures between 5 to –20°C. Doublling time observed at 5°C, 0°C, –10°C and –20°C was 1 day, 3 days, 20 days and about 160 days respectively (Rivkina *et al* 2000), whereas it was 39 days at –10°C in case of another isolate from the Siberian cryopegs (water within permafrost, as briny liquid films and lenses) (Bakermans *et al* 2003). Respiratory activities of bacteria at –20°C in Arctic sea ice have recently been demonstrated with the help of the fluorescent dye 5-cyano-2,3-ditoyl tetrazolium chloride (CTC). Virtually all of the metabolically active cells were found to be associated with particulate matters (Junge *et al* 2004). Occurrence of a bacterium, capable of growing at –12°C, in Arctic sea ice has also been reported. This strain named *Psychromonas ingrahamii*, was found to grow with a generation time of 240 h at –12°C and a generation time of about 12 h at 5°C, which was its optimum temperature for growth. Though metabolic activity of a mixed population of bacteria below –12°C was demonstrated earlier by Rivkina *et al* (2000), this is the first demonstration of growth of an individual bacterium at –12°C (Breeze *et al* 2004).

Using cell-free system, it was shown that both transcriptional and translational machinery of an Antarctic strain of *P. syringae* continued to work at 0°C, albeit in a lower efficiency compared to that observed at its optimum temperature for growth (15–20°C), On the other hand, transcription and translation in cell-free system of *E. coli* ceased to act at 0°C (Ray *et al* 1998). The *hut U* operon, which is involved in the utilization of histidine, was also found to be upregulated at low temperature in the same organism (Kannan *et al* 1998). Earlier, a protease was found to be produced in higher amount at low temperature in the Antarctic yeast *Candida humicola* (Ray *et al* 1992). In view of these two observations and other

evidences available in the literature, it was postulated that enhanced biodegradative activities at low temperature might help the organisms to utilize the scarce nutrients, which are available in the soil of Antarctica (Ray *et al* 1998).

In another investigation, cold-sensitivity (inability to form visible colonies on LB-agar plates at 20°C) of a strain of *E. coli* (D 10) was found to be corrected when it was transformed with a plasmid genomic library, prepared from another strain MC 4100. Subsequent analyses revealed that suppression of the mutation was due to the presence of a gene *bip* A, which encodes a protein, known to be involved in the pathogenicity of enteropathogenic *E. coli*. It belongs to the GTPase superfamily which also includes the elongation factors Ef-G and EF-Tu and the tetracycline resistance-conferring proteins. This study indicated a role of Bip A in cold adaptation (Pfennig and Flower 2001).

6. Role of heat shock proteins

Heat shock proteins (HSPs) are a group of ubiquitously occurring proteins, which are believed to protect the producer organism from thermal stress. Some HSPs appear to help survival and growth of bacteria also at low temperature. It was shown earlier that the high rate of mortality that occurred in a sample of *E. coli* on storage at –80°C for 24 h could be substantially reduced by heating the cell suspension at 42°C for 30 min before cold storage. The inductive synthesis of some known HSPs in the heat-treated samples was also demonstrated (Chow and Tung 1998).

It was also observed that cellular level of another HSP called Clp B, increased 5-6-fold in a cyanobacterial strain *Synechococcus* PCC 7942 when the culture was shifted from 37°C to 25°C. In the absence of this protein, both the growth and photosynthetic activity of a mutant were repressed (Porankiewicz and Clarke1997). Similar findings were subsequently reported by Hossain and Nakamoto (2002) from an investigations on Htp G, which is the prokaryotic homolog of the well-known heat shock protein, Hsp 90. Earlier, the indispensable role of Htp G in *Synechococcus* PCC 7942 for growth at 45°C, was demonstrated by targeted mutagenesis. In this study (Hossain and Nakamoto 2002), the growth of the same mutant at 16°C was found to stop after 20 h, whereas the wild type continued to grow, albeit slowly. When cells were shifted from 30°C to 16°C, photosynthetic activity, measured in terms of oxygen evolution, was reduced to 20% in the cases of wild type and more than 20% in case of the mutant, after two days (the photosynthetic activity of each strain at 30°C was taken as 100%). However, in case of the wild type, the activity was maintained at this level till 5 days, while in the mutant it continued to decrease. Significant induction of Htp G in the wild type strain at 16°C but not in the mutant was evidenced by Western blotting.

Hence it is obvious that both Clp B and Htp G played an important role in cold acclimation of the *Synechococcus* PCC 7942. It has also been known that some molecular chaperones (Hsc B, Hsc 25, trigger factor) are induced by lowering of environmental temperature (Yamanaka 1999; Kawahara *et al* 2000). Though Hsc 66 is a homolog of the heat shock protein HSP 70, its synthesis in *E. coli* was found to be induced not by heat shock but by cold shock (Lelivelt and Kawula 1995). Thus HSPs appear to play a significant role in cold adaptation of bacteria. This is not surprising since HSPs are induced also by a variety of stress conditions other than upshift of temperature. Many of the HSPs are molecular chaperones, which stabilize correct state of folding of the cellular proteins during stress conditions. They also help refolding of the proteins, which are misfolded and solublization of the proteins, which are aggregated. All these corrective functions are called for, both during heat and cold stress.

7. Role of cold shock proteins

Following a downshift of temperature from 37°C to 10°C in mesophilic *E. coli*, synthesis of most of the cellular proteins is repressed for a lag period of 4–5 h. Among the 30–40 proteins, which can be detected during these period by two-dimensional gel electrophoresis, there are some transiently induced proteins called cold-shock proteins (CSPs). These stress proteins have been found to occur both in mesophilic and psychrophilic bacteria. A second class of proteins, called cold acclimation proteins (Caps), is found to be produced exclusively in psychrophilic bacteria. They are overexpressed during prolong growth of the cold-tolerant bacteria at low temperature. The CSPs identified so far in *E. coli* include histone-like proteins, subunit of DNA gyrase, RNA-binding proteins, transcription factor (Nus A), Hsc 66, trigger factor, several acyl lipid desaturases and γ-glutamyl-transpeptidase. The major CSP, CspA, acts as a transcriptional activator or as an *m*-RNA chaperone. Homologs of the *csp A* gene were detected in several Antarctic bacteria (Ray *et al* 1994c). The CSPs are believed to facilitate transcription and translation at low temperature in the mesophilic bacteria. However, the exact role of CSPs in cold adaptation of psychrophiles is yet to be elucidated. A cold acclimation protein (Hsc 25), produced in an ice-nucleating bacterium *Pantoea ananas* KUIN-3, was found to be capable of refolding enzymes, which were denatured by heat, cold and guanidine hydrochloride, but it had higher affinity for cold-denatured enzymes than for heat- denatured enzymes, compared to Gro EL (Kawahara *et al* 2000).

8. Role of RNA degradosome

Degradosome is a protein complex, which is the major determinant factor for the stability of cellular RNA. It

contains several ribonucleases. The degradosome of an Antarctic bacterium *P. syringae*, like that of *E. coli*, has been found to contain an endoribonuclease RNase E and an RNA helicase. But instead of polynucleotide phosphorylase, the exoribonuclease found in *E. coli*, the degradosome of the Antarctic bacterium contains another exoribonuclease, called RNase R. In *E. coli*, this enzyme is known to play an important role in ensuring the quality control of rRNA. The significance of the association of this enzyme with RNase E in the Antarctic bacteria is not definitely known. But it is believed that RNAse R can degrade RNAs with extensive secondary structures. Therefore, by eliminating the necessity of ATP, which is required by helicase, it may help the cell in conservation of energy at low temperature (Purusharth *et al* 2005).

9. Role of cryoprotectants

Cryoprotectants are chemical substances, which are known to accumulate in the body fluids of some wintering frogs and insects. Examples of these substances include sugars (glucose, fructose), sugar alcohols (mannitol, glycerol), and amino acids (alanine, proline). The growth-enhancing effect of glycine betaine, a known osmolyte, on *L. monocytogenes* at low temperature, was demonstrated earlier (Ko *et al* 1994). Other evidences also highlight the cryoprotective role of glycine betaine in bacteria (reviewed by Chattopadhyay 2002a). They are believed to prevent cold-induced aggregation of proteins and maintain an optimum membrane fluidity at low temperature.

10. Formation of viable but nonculturable cells

Many bacteria are known to form a dormant type of cells, which continue respiration and uptake of substrates but cannot be propagated under most or regular laboratory conditions. It is called VBNC state of bacteria. Some scientists believe that it is nothing but a dying sate of bacteria while others opine that it is a strategy for survival under adverse natural environments. Formation of VBNC cells by various bacteria in low temperature environment including Antarctica, is well-documented in literature and hence formation of VBNC cells by various bacteria in low temperature environments including Antarctica (Chattopadhyay 2000).

11. Interlinked adaptive response

A number of evidences are available in the literature indicating association of bacterial cold tolerance with adaptation to various other types of stress conditions (Chattopadhyay 2002b). The role of HSPs in protecting bacteria both under thermal stress and cold stress has already been discussed. A homolog of Hsp 90, called Htp G, which was earlier found to be necessary for cold acclimation of the cyanobacterial strain *Synechococcus* PCC 7942, has also been known to help the same strain to cope with increase in the oxidative stress (Hossain and Nakamoto 2003). The bacterial cryoprotectant glycine betaine also acts as a thermoprotectant in *E. coli* (Caldas *et al* 1999). Glycine betaine and some other low molecular substances (choline, proline) are known as chemical chaperones. They help to stabilize the native state of cellular proteins and thus can help the cell to survive under various stress conditions (Chattopadhyay *et al* 2004). Association between exposure to low temperature and enhancement in the oxidative stress was evidenced in *E. coli* (Smirnova *et al* 2001) and *Listeria monocytogenes* (Liu *et al* 2002). As a matter of fact, in natural environment, the extremophiles are most often exposed to more than one stress conditions at a time. The thermophiles in addition to high temperature, have to tolerate high salinity and desiccation. The piezophilic organisms have to survive high pressure and high or low temperature. The isolation of a strain of Cyanobacteria, resistant to ionizing radiation, from a hypersaline evaporation pond of Antarctica (Billi *et al* 2000) and isolation of three strains of *Deinococcus*, tolerant to low temperature, desiccation and ultraviolet ray, from continental Antarctica (Hirsch *et al* 2004), bolster the concept of interlinked stress resistance of bacteria.

12. Role of antifreeze proteins

The role of antifreeze proteins (AFPs) in preventing freezing of blood of fishes in low-temperature environment is well-known. So far five types of AFPs have been detected in fishes. In addition to that, occurrence of two types of AFPs in insects and six types of AFPs in different plants has also been reported (Gilbert *et al* 2004). In freeze-avoiding organisms (e.g. primarily fishes) the AFPs act by promoting supercooling of the body fluids at subzero temperatures. This is called thermal hysteresis (TH). On the other hand, in freeze-tolerant organisms (e.g. some plants and insects), the AFPs inhibit the re-crystallization of the extracellular ice crystals. The role of AFPs in bacterial cold adaptation has started coming to the limelight during the past few years. The presence of thermal hysteresis proteins in bacteria was demonstrated for the first time by Duman and Olsen (1993) and a strain of *Moraxella* sp. was the first example of an Antarctic bacterium that was found to produce an AFP (Yamashita *et al* 2002). The demonstration of AFPs in 11 bacterial isolates obtained from several Antarctic lakes (Gilbert *et al* 2004) and a Ca^{2+}-dependent AFP in an Antarctic isolate *Marinomonas primoryensis* (Gilbert *et al* 2005) underscores the importance of the AFPs for the survival of bacteria in extreme cold environment.

Most of the bacterial AFPs, known so far, are characterized by substantially lower TH value compared to that of AFPs, isolated from animals. Hence the strategy, adopted by the bacteria, which produce them for adaptation to low temperature, is freeze-tolerance and not freeze avoidance. In contrast to this, the recent report by Gilbert *et al* (2005) presents the first evidence of an effective freeze-avoidance strategy in a bacterium. The AFP, mentioned in this report, showed hyperactive thermal hysteresis. Unlike many other bacterial AFPs, which are found to be exported out of the cell, the AFP activity in this case was found to be present only in the supernatant of cellular lysate. Hence it was suggested by the investigators that the protein might be localized to the periplasmic space. It is also unique among the other bacterial AFPs in having dependence on Ca^{2+}. Hence discovery of this AFP appears to be a significant addition to the state of knowledge on bacterial AFPs.

13. Other strategies

A couple of years ago, using selective capture of transcribed sequences (SCOTS, a novel procedure for differential clon-ing of c-DNA) Liu *et al* (2002) identified several RNAs, which were expressed differentially in *Listeria monocytogenes* by a downshift of temperature from 37°C to 10°C. The transcripts, the level of which was increased at low temperature, included (besides those already mentioned) m-RNAs encoding, a flagellar protein, an alternative sigma factor, a transcriptional antiterminator, a repressor protein, three chaperone proteases, a cysteinyl-tRNA synthetase, products related to the biosynthesis of tryptophan and histidine, a fibronectin binding cell-surface protein, products related to two proteins involved in the degradation of cellulose and ethanolamine, malolactic enzymes and some proteins with unknown function unique to *L. monocytogenes*.

14. New isolates

Recent isolation and characterization of a strain of *Pedobacter himalayensis* from a water sample of the snout of Hamta glacier in the Himalayan mountain range of India (Shivaji *et al* 2005a) and a number of novel Antarctic species belonging to the genus *Psychrobacter* (Shivaji *et al* 2004, 2005b), *Pseudonocardia* (Prabahar *et al* 2004), *Halomonas* (Reddy *et al* 2003a), *Sporosarcina* (Reddy *et al* 2003b), *Leifsonia* (Reddy *et al* 2003c), *Planococcus* (Alam *et al* 2003) and *Pseudomonas* (Reddy *et al* 2004) have furthered the scope of research in the area of bacterial cold adaptation. Investigations on various aspects involving these organisms are likely to generate more information on the mechanism of cold adaptation, in near future.

15. Conclusion

It is obvious from the foregoing discussion that studies conducted so far have considerably expanded the horizon of our knowledge on the mechanism of bacterial cold-tolerance. The nature of the cold-active enzymes and various metabolic pathways, which are found to be upregulated at low temperature, are revealing as to the modus operandi of bacterial physiology at low temperature. The implication that tolerance to different types of stress conditions is an interlinked phenomenon, hints at the intricacy of cellular machinery. The discovery of antifreeze proteins in bacteria provides an impetus to this area of research. However the role of small molecules (cryoprotectants) in cold adaptation of bacteria remains unexplored by and large though it is well-studied in frogs, insects and plants. Investigations on this aspect may not only offer some more clues to the mechanism of cold adaptation but also help in exploiting the potential of these compounds to be used in cryopreservation of tissues and organs.

Mutants of cold-tolerant bacteria, which are unable to grow or which grow slowly compared to the wild-type strain at low temperature, are valuable tools for elucidation of the molecular basis of bacterial cold adaptation. The role of two cold-sensitive mutants in revealing the essential role of an anteiso saturated fatty acid (a-$C_{15:0}$) in cold adaptation of *L. monocytogenes* was mentioned earlier (Annous *et al* 1997). In a cold-sensitive mutant of the Antarctic strain *P. syringae*, the transposon-disrupted gene was found to be a homolog of the *recD* gene. Evidences obtained by this investigation also indicated that the RecD protein might be involved in the DNA repair process at low temperature (Regha *et al* 2005). Some other biochemical mutants have recently been isolated in CCMB (unpublished). Cold-sensitive mutants, which are sensitive to more than one type of stress conditions, are likely to suggest some hitherto unknown mechanism of stress adaptation.

One of the most desirable targets in investigations on bacterial cold adaptation is genetically engineered strains that are capable of degrading man-made wastes in extreme cold environments. The recent observation that a mesophilic strain of *E. coli* could be grown at low temperature by expressing two chaperonin genes, obtained from an Antarctic bacterium, is a major breakthrough in this area (Ferrer *et al* 2003). Spillage of petroleum products on Antarctic soil due to human activities, is a growing menace to the environment, which was pristine once upon a time. Bioremediation of these pollutants using recombinant strains is a major challenge at present to the scientists.

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