Mini-review

Metabolism in bacteria at low temperature: A recent report

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The adaptability of bacteria to extreme cold environments has been demonstrated from time to time by various investigators. Metabolic activity of bacteria at subzero temperatures is also evidenced. Recent studies indicate that bacteria continue both catabolic and anabolic activities at subzero temperatures. Implications of these findings are discussed.

[Sengupta D and Chattopadhyay MK 2013 Metabolism in bacteria at low temperature: A recent report. *J. Biosci.* **38** 409–412] **DOI** 10.1007/s12038-013-9306-y

1. Bacteria at low temperature environments

It is well known that bacteria as well as various other forms of life survive and thrive optimally in moderate conditions of temperature, pressure, pH, salinity and other environmental parameters. However, occurrence of live bacteria in extreme environments is well documented. Bacteria were found to exist in the very acidic river Rio Tinto and the driest Atacama Desert. Live bacteria were detected in subzero environments like permafrost and even at a depth of 3603 m in Lake Vostok, below the overlaying glacial ice (Priscu *et al.* 1999). Some of the cold-adapted organisms occur in polar ice, where the temperature reaches nearly 28°C during warm season. Needless to say, it is the flexibility of their cellular machinery that enables them to cope with the seasonal fluctuations. On the other hand, some others evolve as true psychrophiles, which are unable to grow at higher temperature. *Psychrobacter ingrahamii* was found to survive in a sea ice column within a temperature range of −1.8°C to −30°C at Elson Lagoon, Pt. Barrow, Alaska (Breezee *et al.* 2004). Occurrence of cold-adapted bacteria is not confined in natural environments. Some food-borne pathogens (e.g. *Listeria monocytogenes*) survive in the frozen food compartments.

2. Clues to the mechanism of cold tolerance

During the past few decades, extensive investigations in various laboratories led to the generation of a number of clues to the basis of cold tolerance in bacteria (Chattopadhyay 2006). At low temperature, bacteria are challenged with a number of oddities caused by reduction in the rate of biochemical reactions that sustain the life. Decrease in fluidity hinders the normal functioning of the cell membrane. Bacteria isolated from extreme cold environments were found with increased branched chain, short chain, anteiso and unsaturated fatty acids. They were also found to synthesize more *cis* fatty acids in preference to *trans* fatty acids. All these factors are known to contribute to increase in membrane fluidity. Enhanced synthesis of some polar carotenoids was postulated to play a significant role in homeoviscous adaptation of membrane fluidity in two Antarctic bacteria (Chattopadhyay and Jagannadham 2001). In order to adjust with the low enthalpy and the reduced atomic and molecular motions at low temperature, the cold-adapted proteins attain conformational flexibility achieved through reduction in strength and number of non-covalent interactions, like the hydrophobic bond. This stabilizes the folded and biologically active conformation. In general the overproduction of heat shock proteins (Gro EL/ES) leads to reduced ability of *E. coli* to survive at low temperature. On the other hand, adaptation of the same organism to low temperature is associated with suppression of heat shock protein synthesis and increased production of cold shock proteins like trigger factor, which contributes to the improved viability of the organism at low temperature (Kandror and Goldberg 1997). However, some heat shock proteins (Clp B, Htp G) were found to be essential both for thermotolerance and cryotolerance of the cyanobacterium *Synechococcus* PCC

Keywords. Anabolism; bacteria; catabolism; cold adaptation

Published online: 26 February 2013

7942. Inductive synthesis of Dna K/Dna J upon heat treatment was associated with significantly improved survival of *E. coli* in frozen storage conditions (reviewed in Chattopadhyay 2008). A high level of post-transcriptional modification of t-RNA by dihydrouridine also has a major role in psychrophiles. Dihydrouridine disturbs the stacking that stabilizes the RNA. Psychrophilic bacteria are also found with more of the less stable A:U base pairing compared to the more stable G:C pair (Feller 2007; Cipolla *et al.* 2011). Sensing the change in the environmental temperature is a crucial requirement for adaptation to cold environments. The role of a membrane protein and lipopolysaccharide in sensing the change was demonstrated using an Antarctic bacterium *Pseudomonas syringae* (Ray *et al.* 1998).

3. Availability of liquid water

Presence of liquid water is indispensable for sustenance of life. The amounts of unfrozen water present by weight in soil samples collected from permafrost were found to vary between 5% and 6% at −1.5°C, 2% and 3% at −10°C, and 1% and 2% at temperatures −15°C and −20°C (Rivkina *et al.* 2000). Using a water-content reflectometry probe at subzero temperatures above −20°C, Jakosky *et al.* detected abundant liquid water that was capable of harbouring microbes and permitting transport of both nutrients and waste products by diffusion. Below this temperature, liquid water remained as adsorbed water, which also supports diffusion of ions (Jakosky *et al.* 2003). The thickness of films was 15 nm at −1.5°C, whereas it decreased to 5 nm at −10°C (Rivkina *et al.* 2000).

4. Microenvironments in ice

Access to nutrients is limited by the thickness of the films. Low levels of nutrients present in the frozen environments remain concentrated in these narrow channels of unfrozen water. Habitable brine-filled pores with both isolated and connected brine tubes and veins were observed in Arctic wintertime sea ice at −20°C (Junge *et al.* 2004). Using a scanning electron microscope (SEM) equipped with a cold stage and an energy-dispersive X-ray analyser, the presence of approximately 2.5 M sulphuric acid was demonstrated in a region where the cross section of the vein was 1 μm^2. It was also shown that hydrochloric acid also could concentrate in veins. Using Raman spectra, Fukazawa and his co-workers evidenced the presence of aqueous solutions of sulphuric and nitric acids in the veins of Antarctic ice. These acids are the major electron acceptors for bacteria in ice. It was postulated that bacteria, in these strongly acidic environments, must have some proton pumps or low proton membrane permeability in order to maintain their interior pH around neutrality. In the same way, bacteria should be capable of adapting to the environment if there is higher concentration of salts in the veins (Price 2000).

5. Metabolism in cold environments

Accumulating evidences indicate that bacteria not only remain alive in ice but also continue metabolism. *Psychromonas ingrahamii*, incubated in saline broth media at −12°C in ethylene glycol bath, was found to grow with a generation time of 240 h, or 10 days. It was found to grow with a generation time of 12 h at an optimum temperature (5°C) (Breezee *et al.* 2004). Metabolic activities of living cells, estimated by reduction of resazurin, were observed at −10°C in Siberian permafrost samples, which had been frozen for around 43,000 years. Most of the non-spore-forming bacteria reduced resazurin at the rate of 0.6 fmol $cell^{-1}\,day^{-1}$. The constant resazurin reduction rate till day 75 indicated that the cells were still metabolically active. Among many isolates, *Psychrobacter cryopegella* had a detectable growth rate of 0.016 day^{-1}. It was also found that the cells grew most efficiently at 4°C and the energy required per cell division increased dramatically below this temperature (Bakermans *et al.* 2003). Generation time also was increased with decreased nutrient concentration. Incorporation of ^{14}C-labelled acetate by the native bacterial population in Siberian permafrost, as studied by Rivkina *et al.* (2000), showed that the doubling times at 5°C, 0°C, −1.5°C, −10°C, −15°C and −20°C were 1, 3, 6, 20, more than 40 and 160 days, respectively. At temperatures between 0°C and −10°C, the stationary phase was reached in 200 to 350 days. They also suggested that in nature, bacteria were in the stationary phase, which was reached in less than 1 year after freezing (Rivkina *et al.* 2000). Respiratory activity associated with particulate matter in Arctic sea ice at −20°C was successfully identified using the fluorescent dye 5-cyano-2,3-ditoyl tetrazolium chloride (CTC) (Junge *et al.* 2004). The cell-free translational and transcriptional machinery of an Antarctic strain of *P. syringae* continued to work at 0°C, whereas it ceased to work at the same temperature in *E. coli* (Ray *et al.* 1998). Adenylate concentration was found to increase at temperatures below freezing, indicating elevation of total energy in the cell (Amato and Christner 2009). Earlier, ice wedges were thought to be an inhospitable environment for sustenance of life. However, a recent investigation demonstrated mineralization of radio-labelled acetate at 5°C and *in situ* CO_2 flux from the Canadian ice wedge Axel Heiberg (Wilhelm *et al.* 2012).

Some time back, both catabolic and anabolic activities of bacteria at subzero temperatures were reported for the first time by Drotz *et al.* (2010). They used soil samples collected from the surface layer from a site in northern Sweden (64° 11′ N, 19°35′ E). A ^{13}C glucose solution was added to samples maintaining a constant ratio with the amount of soil organic matter (SOM) present. Four temperatures for

incubation were chosen, viz. +9°C, +4°C, −4°C and −9°C, at which the experiments were continued for 6, 10, 99 and 160 days, respectively. To measure non-biological production of [^{13}C] carbon-dioxide gas (CO_2), control samples were sterilized with 0.5% sodium azide. The CO_2 content of the headspace sample gas was analysed by gas chromatography-isotopic ratio mass spectrometry (GC-IRMS) considering the known amount of CO_2 at natural ^{13}C abundance as control. Production of CO_2 was found to increase slowly with time, as observed till the end of incubation time at all temperatures. The authors commented that the previous investigators might have underestimated the potential for CO_2 production at freezing temperatures by limiting the incubation time to days or weeks.

The total pool of newly synthesized ^{13}C compounds was taken as a measure of total microbial synthesis. For identification of ^{13}C compounds, the chemical shifts, intensities and coupling patterns of the ^{13}C magic-angle spinning NMR spectra were compared to these parameters in the previous reports. Glycerol, phospholipids, polymeric carbohydrates like glycogen, protein compounds and ethanol were identified by NMR. The fatty acids were identified as phospholipid dioleoylphosphatidylcholine. Formation of both glycerol (an antifreeze agent and also used as substrate by some microorganisms) and ethanol was detected +9°C and +4°C. At −4°C, significant levels of glycerol formation was observed from day 34 till the end of incubation. Although at an insignificant level, glycerol was also found to be formed at −9°C at the end of the incubation period. Glycogen signals were obtained at +9°C. The time corresponding to the consumption of 50% of the added glucose was 2.5 days at +9°C, 2.9 days at +4°C, and 93.7 days at −4°C. At this point the proportion of synthesized [^{13}C]CO_2 and organic ^{13}C compounds showed no significant difference at different temperatures of incubation. The carbon use efficiency index and growth index also showed similar values for all temperatures. The authors suggested that the shift in the lipid composition of cell membrane might explain the lag phase in the frozen samples.

It was also found that the degree of unsaturation and length of acyl chains in the membrane lipids was more at −4°C when compared to those at +4°C and +9°C. The synthesis of phospholipids in the synthesized ^{13}C compounds was also higher at −4°C compared to that at other incubation temperatures. Active synthesis of the cell membrane fatty acids corroborated the findings of other studies in which total biomass was found to be higher in snow-covered soil than that observed in soil at higher temperatures.

6. Implications

This investigation clearly indicates that both catabolic and anabolic processes continue at typical wintertime soil temperatures, thus nullifying the postulation made by the previous investigators that catabolic processes predominate at subzero temperatures over anabolism. The report significantly contributes to the present state of knowledge on life at extreme temperature. The lead provided by the studies may find useful application in bioremediation at extreme cold environments and help us further in exploring the possibilities of occurrence of life in frozen planets.

References

Amato P and Christner BC 2009 Energy metabolism response to low-temperature and frozen conditions in *Psychrobacter cryohalolentis*. *Appl. Environ. Microbiol.* **75** 711–718

Bakermans C, Tsapin AI, Souza-Egipsy V, Gilichinsky DA and Nealson KH 2003 Reproduction and metabolism at −10°C of bacteria isolated from Siberian permafrost. *Environ. Microbiol*. **5** 321–326

Breezee J, Cady N and Staley JT 2004 Subfreezing growth of the sea ice bacterium "*Psychromonas ingrahamii*". *Microb. Ecol*. **47** 300–304

Cipolla A, D'Amico S, Barumandzadeh R, Matagne A and Feller G 2011 Stepwise adaptations to low temperature as revealed by multiple mutants of psychrophilic α-amylase from Antarctic bacterium. *J. Biol. Chem.* **286** 38348–38355

Chattopadhyay MK 2006 Mechanism of bacterial adaptation to low temperature. *J. Biosci*. **31** 157–165

Chattopadhyay MK and Jagannadham MV 2001 Maintenance of membrane fluidity in Antarctic bacteria. *Polar Biol.* **24** 386–388

Chattopadhyay MK 2008 Cryotolerance in bacteria : Interlink with adaptation to other stress factors. *Trends Microbiol.* **16** 455

Drotz SH, Sparrman T, Nilsson MB, Schleucher J and Oquist MG 2010 Both catabolic and anabolic heterotrophic microbial activity proceed in frozen soils. *Proc. Natl. Acad. Sci. USA* doi/10.1073/pnas.1008885107

Feller G 2007 Life at low temperature: is disorder the driving force? *Extremophiles* **11** 211–216

Jakosky BM, Nealson KH, Bakermans C, Ley RE and Mellon MT 2003 Subfreezing activity of microorganisms and the potential habitability of Mars' polar regions. *Astrobiology* **3** 343–350

Junge K, Eicken H and Deming JW 2004 Bacterial activity at −2 to −20°C in Arctic wintertime sea ice. *Appl. Environ. Microbiol.* **70** 550–557

Kandror O and Goldberg AI 1997 Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperature. *Proc. Natl. Acad. Sci. USA* **94** 4978–4981

Price PB 2000 A habitat for psychrophiles in deep Antarctic ice. *Proc. Natl. Acad. Sci. USA* **97** 1247–1251

Priscu JC, Adams EE, Lyons WB, Voytek MA, Mogk DW, Brown RL, McKay CP, Takacs CD, *et al*. 1999 Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. *Science* **286** 2141–2144

Ray MK, Kumar GS, Janiyani K, Kannan K, Jagtap P, Basu MK and Shivaji S 1998 Adaptation to low temperature and regulation of gene expression in Antarctic psychrotrophic bacteria. *J. Biosci.* **23** 423–435

Rivkina EM, Friedmann EI, McKay CP and Gilichinsky DA 2000 Metabolic activity of permafrost bacteria below the freezing point. *Appl. Environ. Microbiol*. **66** 3230–3233

Wilhelm RC, Radtke KJ, Mykytczuk NCS, Greer CW and Whyte LG 2012 Life at the wedge: the activity and diversity of Arctic ice wedge microbial communities. *Astrobiology* **12** 347–360

MS received 16 August 2012; accepted 28 January 2013

Corresponding editor: B Jagadeeshwar Rao