

Molecular Mechanisms of Cold Adaptation in Bacteria

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Abstract

Bacteria are ubiquitous in nature and have the ability to survive in severe conditions of low temperature, high pressure and often changing osmotic environment. However, mechanisms of survival of bacteria in extreme environments are still not completely understood. Low temperature adaptation in bacteria is initiated by its abilities to sense the change in environmental temperature and transduce the signal to regulate the expression of required genes for survival. Adaptation at low temperature involves several strategies such as increase in membrane fluidity, decrease in activation energy for enzyme activity, production of cold shock chaperones, induction of cold inducible genes, inhibition of the cold denaturation of protein and by enhancing the efficiency of transcription and translation. This review highlights the molecular mechanisms of cold adaptation in bacteria.

Keywords: Cold adaptation; Membrane fluidity; Cold inducible Gene; Cold active enzyme

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Introduction

Bacteria have the ability to survive in severe conditions of low temperature, high pressure and often changing osmotic environments. Psychrophiles serve as excellent models for

understanding the molecular mechanisms of cold adaptation [1-9]. Psychrophilic microorganisms are able to proliferate in low temperature conditions by altering the fluidity of membrane, maintaining the stability of DNA and transcriptional machinery and their unique ability to maintain enzymatic reactions [6, 8, 10, 11].

A number of genes such as *hns*, a DNA binding protein H-NS [12, 13], *csdA*, a DEAD-box helicase [14], *pnp*, a polynucleotide phosphorylase [15], *OppA*, a oligopeptide-binding protein [16], *rnr*, a RNase R [2, 3] *TrmE*, a t-RNA modification GTPase [6, 7, 10], AAT, an aspartate aminotransferase [9] and *recD*, a DNA recombination and repair gene [4, 8, 17-19] have been implicated in cold adaptation. Several genes such as *cspA* in *Escherichia coli* [20], *csdA* in *E. coli* [21], *cspB* in *E. coli* [22], *cspG* in *E. coli* [23], *cspI* in *E. coli* [24], *deaD* in *Methanococcoides burtonii* [25], *clpX* (ATPase subunit of Clp protease) in *Lactococcus lactis*, *icl* (isocitrate lyase) from *Colwellia maris* [26], *hutU* in *Pseudomonas syringae* (Lz4W) [27], *rpoS* in *Pseudomonas putida* [28], *trmE* in *Pseudomonas syringae* (Lz4W) [6] and *icd* (isocitrate dehydrogenase) from *Pseudomonas psychrophila* [29] are up regulated during the cold shock and play important role in cold adaptation. RNA polymerase of *P. syringae* (Lz4W) (Antarctic bacterium) has a special ability to preferentially transcribe cold inducible genes at low temperature [11]. At low temperature, expressions of cold inducible genes are regulated under the influence of a cold inducible promoter [6, 7, 27, 30]. Significant improvements in understanding the mechanisms of bacterial cold adaptation have been achieved. This review

highlights recent development in understanding the molecular mechanisms of cold adaptation in bacteria.

Molecular Mechanisms of Cold Adaptation

Role of Membrane Fluidity

At low temperature, bacterial proliferation is dependent on an ability to restore membrane fluidity to maintain the membrane functions such as transport, energy production, signal transduction, cell growth and cell division. Cold shock is known to enhance the rigidity of membranes and bacteria modulate membrane fluidity in several ways by changing the polar head groups of glycerophospholipids which alters their packing in the membrane bilayer [31], or by changing the magnitude of fatty acid desaturation [32-35], or by changing the chain length of fatty acids [36], or by changing the ratio of *iso* and *anteiso* fatty acid [37, 38] and or by changing the proportion of *cis* and *trans* fatty acid [39] as common modes. It has also been observed that certain genera evolved a preferential mechanism for overcoming the membrane rigidity associated with low temperature adaptation. The Eicosa Pentaenoic Acid (EPA), present in membranes as a component of phospholipids regulates the membrane fluidity at low temperature. Antarctic bacterium, *Shewanella livingstonensis* Ac10 produces Eicosa Pentaenoic Acid (EPA) when grown at 4°C. In contrast, EPA-less mutants are cold sensitive, filamentous and exhibit multiple nucleoids phenotype and display defective cell division at low temperature [40].

Role of Fatty acid Desaturation

Biological membranes exist in liquid crystalline phase and change to a gel-phase upon low temperature exposure. The alteration in proportion of fatty acids (saturated to unsaturated) could effectively alter the membrane fluidity. In response to low temperature *Escherichia coli* regulates its membrane fluidity by enhancing the amount of *cis*-vaccenic acid by elongation of palmitoleic acid [41].

A higher proportion of unsaturated fatty acid has been reported in psychrophilic bacteria as compared to mesophilic bacteria [42]. Some bacteria adapt to low temperature stress by increasing amount of unsaturated fatty acids being incorporated

into the membrane phospholipids [43, 44]. The saturated fatty acids are known to decrease membrane fluidity due to tight packing of acyl chains of the saturated fatty acids. Whereas, unsaturated fatty acids are known to enhance the fluidity due to poor packing and kinks caused by the presence of *cis* double bonds in unsaturated fatty acid [36, 45].

It has been demonstrated that in response to low temperature, *Bacillus subtilis* and *B. megaterium* convert existing saturated fatty acids into unsaturated fatty acids [46-48]. The conversions of these fatty acids are catalyzed by enzyme desaturase (encoded by the *des* gene) which is transiently induced upon downshift in temperature [49, 50]. The cold induction of *des* gene is regulated by two component signal transduction consisting of a membrane associated sensor Kinase (DesK) and a cytoplasmic response Regulator (DesR) [51]. The cold inducible desaturase has been reported in the non-photosynthetic bacterium (*Bacillus* sp.) [49]. In *Bacillus subtilis*, cold-shock responses were analyzed using DNA microarrays and results revealed that *des* is the strongest cold inducible gene [52]. The deletion of *des* gene and the absence of isoleucine cause a severe cold sensitive phenotype. There are reports that *des* expression can replace the mechanisms of isoleucine dependent fatty acid branching during cold adaptation [53].

Role of Anteiso-branched fatty acids

Incorporation of branched chain fatty acids (*iso* and *anteiso*) into lipid bilayer exerts a fluidizing effect on the membrane. In *Bacillus subtilis* and *Brevibacterium fermentans*, the increase in *anteiso*-branched chain fatty acids (methyl branching from the third last ante-penultimate carbons in the chain) were observed upon lowering the growth temperature and concomitant decrease in *iso*-branched chain fatty acids (methyl branching at second last carbon of fatty acid) were also observed [54]. *Listeria monocytogenes* (food borne pathogen) has the ability to grow at refrigerated temperature and predominantly synthesized α -C15:0 to maintain the homeoviscous viscous adaptation in response to cold adaptation [55].

Role of cis and Trans fatty acids

The isomers (*cis* and *trans*) of fatty acids differently affect membrane fluidity [56, 57]. The *cis*-unsaturated fatty acids are responsible for lowering the phase-transition temperature [58] and *trans*-unsaturated fatty acids are also known to lower the phase transition temperature but to a lesser extent. In gram-negative bacteria, *trans*-monounsaturated fatty acids were observed to be predominant [59] and are synthesized by direct isomerization from existing *cis* unsaturated fatty acid to *trans* unsaturated fatty acids [60-62]. The increased content of *trans*-fatty acid was observed with increase in growth temperature [37-39] or with solvent [37-39, 63-65] or exposure to a salt shock [66].

Transcription and Translation

Psychrophilic microorganisms have the unique ability to transcribe and translate at low temperatures [11]. The efficiency of transcription at low temperature is retarded by increased stability of DNA duplex (enhanced negatively super coiled), low efficiency of promoter melting by RNA polymerase and slow diffusion of the enzyme and substrates. The mechanisms by which RNA polymerase overcomes these barriers in psychrophiles are poorly understood. But, there are reports available that RNA polymerase of psychrophilic *Pseudomonas syringae* (Lz4W) is active at low temperature [11]. RNA polymerase of psychrophilic bacteria also have a typical eubacterial composition consisting of β , β' , α and σ subunits; and prefers super coiled DNA for transcription of genes at low temperatures. Gyrase A is known to be associated with RNA polymerase and help in the unwinding of DNA during transcription at low temperature.

Cold shock arrests the transient growth of bacteria as a result of blockage in translation due to highly stable secondary structure of mRNAs at low temperature [67, 68]. The gene *yfiA* encode for Protein Y (PY) which is a cold shock protein in *Escherichia coli* and mediates major protein synthesis repression during cold shock [69]. The cold inducible genes are reported to have longer than 100 bp of UnTranslated Region

(5'-UTR) in different bacteria and cyanobacteria [6, 10, 25, 70]. In *Escherichia coli*, 5'-UTR (*cspA*, *cspB* and *csdA*) over-expression and following cold shock result in de-repression (prolonged synthesis) of the respective genes [70, 71]. The cold-box containing genes are reported to be regulated by efficient transcriptional and translational machinery during cold-shock [72-76]. The cold-box element functions as a putative repressor binding site [24, 70]. The posttranscriptional regulator such as CspA family of cold shock proteins has the potential to destabilize the secondary structures of mRNA to allow efficient translation at low temperatures [77].

Role of Protein Folding

The newly synthesized nascent polypeptide must be precisely folded (tertiary and quaternary structures) before becoming fully functional. The mesophilic organisms exposed at low temperature induce transient over-expression of selected proteins. The low temperature exposure of bacteria initially induces preferential synthesis of some proteins known as Cold-shock proteins (Csps) to regulate various cellular processes [78]. Several Csps are known to regulate the synthesis of unsaturated fatty-acid and has already been identified in *Desulfotalea psychrophila* genome [79], *Colwellia psychrerythraea* 34H [80] and *Pseudoalteromonas haloplanktis* [81]. The molecular chaperones like caseinolytic proteases (Clps), Trigger Factor (TF), GroEL, DnaK and GroES [78, 82, 83] are up regulated in *E. coli* during cold shock. It has already been established that folding and refolding of cold-damaged proteins is crucial during cold adaptation [84]. The chaperones like the CaseinoLytic proteases (Clps) [85-87], Trigger Factor (TF) [84], GroEL and GroES [88] and Hsc66 molecular chaperone in *E. coli* [89] have been implicated in cold adaptation. The molecular chaperones prevent aggregate formation by binding to exposed hydrophobic regions of unfolded polypeptides. It has also been proposed that chaperones and nascent polypeptide interact together to regulate the co-translational protein folding [90]. A previous study has confirmed that *cpn60* (encoding GroEL) and *cpn10* (encoding GroES) of the Antarctic bacterium *Oleispira antarctica*, when expressed in *trans*, could support the growth of *E. coli* at 4°C,

demonstrating the importance of chaperone-mediated protein folding during growth at low temperature [91].

Cold Adapted Enzymes

The cold adapted enzymes are well known to play an important role for low temperature adaptation of bacteria [6, 9, 10]. The presence of cold adapted enzymes in psychrophilic organisms helps them to compensate for the drastic reduction in biochemical reaction rates induced by low temperatures. In order to cope up with this constraint, psychrophilic organisms enhance catalytic efficiency by increasing K_{cat} and decreasing K_m , or by changing both parameters as an adaptive response to low temperature [92, 93]. The presence of high specific activities of psychrophilic enzyme at low temperature is already reported in the literature [91, 94, 95]. Most cold adapted enzymes are thermo labile [6, 10, 96, 97] and exhibit optimum activity at $<20^{\circ}\text{C}$ [91]. The psychrophilic enzymes isolated from different sources also exhibit drastic differences in optimum temperature for their activity (Eg. psychrophilic monomeric isocitrate dehydrogenases isolated from *Colwellia maris* shows optimum activity at much lower temperature than that isolated from *Colwellia psychrerythraea* [98]). Attempts are also ongoing to identify psychrophilic alkaline proteases from cold adapted bacteria which may have great utility in the detergent industry.

Conclusions

Cold adaptation is initiated by the ability of bacteria to sense a change in environmental temperature and transduce signal to regulate the expression of essential genes for survival. The cold inducible genes, cold active enzymes and genes of various metabolic pathways are known to be up regulated at low temperature and play a critical role in cold adaptation. Increased understanding of molecular mechanisms of cold adaptation would be helpful to construct genetically engineered bacterial strains which can degrade man-made waste in extreme cold environments.

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