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Growth and Survival of *Pseudomonas* Species Under Cold Shock

Rashed Noor*, Tasmin Tabassum, Tahsin Tabassum, Nafisa Tabassum and Syeda Muntaka Maniha

School of Life Sciences (SLS), Independent University, Bangladesh (IUB), Aftabuddin Ahmed Road, Bashundhara, Dhaka, Bangladesh

***Corresponding Author:** Rashed Noor, School of Life Sciences (SLS), Independent University, Bangladesh (IUB), Aftabuddin Ahmed Road, Bashundhara, Dhaka, Bangladesh.

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Abstract

The *in vitro* simulations of bacterial growth and survival against numerous stress factors have shown significant survival strategies within the bacterial cells. While heat stress and the oxidative stresses have been analyzed rigorously on *Escherichia coli, Bacillus* spp., *Salmonella* spp.; and on *Pseudomonas* spp., the impact of cold shock is comparatively less understood so far. Present review discussed the possible survival strategies based on the *Pseudomonas* strains against temperatures lower than the ambient one required for normal cellular homeostasis. As a ubiquitous microorganism, it would be interesting to know about the cold tolerance of Pseudomonas aeruginosa, *P. fluorescence*, or *P. putida*. Upon cold shock, the rise in the possible dead cells and the viable but non-culturable (VBNC) cells would be not unlikely. Besides, the molecular mechanisms underlying the probable survival against the cold shock would add new insights to the existing knowledge on the bacterial stress management.

Keywords: *Pseudomonas* Spp; Cold Shock; Culturable Cells; Viable But Non-Culturable (VBNC) Cells

Background

A range of fascinating works have been conducted so far to detect bacterial growth and survival with (i) different simulated variables (temperature, oxidative stress, pH, rate of aeration, nutrient variability, differing redox potentials and water activity, in presence of varieties of toxic chemicals like hydrogen peroxide or ethanol, various stabilizers, etc.), (ii) conditions to especially detect the growth kinetics along with the bacterial generation time, (iii) to monitor the bacterial survival and culturability, (iv) the viable but non-culturable (VBNC) cells under various stressed conditions [1-10]. During such *in vitro* experiments, a wide range of bacterial species have been shown to activate different transcriptional regulatory network (TRN) as the defensive strategy especially at the early stationary phase in response to the stress factors [7,11]. Our earlier reports showed that upon heat shock and the oxidative stress, a huge portion of the cells of *Escherichia coli* viable but non-culturable (VBNC) at the entry of the stationary phase (induced by the accumulation of the reactive oxygen species (ROS) along with the involvement of rpoS gene encoding σ^S, the general stress responsive sigma factor [9,10,12,13]. The VBNC cells are expected to undergo σ^E-specific lysis suggestive of providing nutrients for the remaining surviving population [4,7]. Such an observation may be further interesting to focus on the bacterial growth under cold shock state in order to detect the surviving cells. However, this is to be noted a few study have already reported the impact of cold shock especially on *E. coli*, and a few species of *Bacillus* and *Pseudomonas* [14,15-17] which eventually raises the interest to detect the exact molecular mechanism underlying the cold shock response of bacterial cells.

The study of cold-shock response is now in the limelight because of its commercial and health implications. Research on microbial growth and survival under cold-shock appears to be useful especially for the avoidance of the potentially disastrous situations in various food industries including the cold storages. Understanding cold-shock response of food-borne pathogens such as *Listeria* is imperative since refrigeration is a commonly used method of food storage. Cells, which are cold-shocked prior to freezing, may exhibit better cryotolerance. Another aspect of studying the cold shock response of bacteria is that certain proteins which cannot produced

efficiently at 37°C may be initially overproduced in large quantities at low temperatures using cold-inducible promoters, for example, the promoter of *csp* A, encoding the major cold shock protein of *E. coli*. Such strategy is powerful tool in microbial biotechnology as well possesses medical importance too. Along these lines, current review discussed the facets of cold shock response including the molecular aspects especially within the ubiquitous *Pseudomonas* cells.

Study of heat shock versus cold shock

According to the type of temperature change during *in vitro* simulations, the bacterial response has been classified into two categories: (i) the heat shock response (HSR) and (ii) the cold shock response (CSR). During the HSR, principally the heat shock sigma factors (σ^H, σ^E and σ^D) and the chaperons (GroEL, DnaK-J, ClpX, etc.) work in a concert [6,7,9,10] while during cold shock, nucleic acid structure and proteins interacting with the biological information molecules DNA and RNA appear to play a major cellular role [14,18]. A number of physiological changes especially in *E. coli* and *B. subtilis* have been noted in response to temperature downshift including the drop in the membrane fluidity, malfunctioning ribosome, transcription and translational defects along with inefficient folding of some proteins [15]. Misfolding of proteins and aggregation of misfolded peptides are major problems at high temperatures [7]. As stated above, cells have heat-shock-inducible systems to synthesize heat- shock proteins which as molecular chaperones (GroEL, GroES) by assisting in correct protein folding and proteolysis of abnormally folded polypeptides. A peptidyl prolyl isomerase catalyzing the cis/trans isomerization of peptide bonds in *E. coli*, is induced upon cold shock at a modest level after a growth lag period of 2-3 h. Similar to other cold shock proteins, its synthesis is induced after temperature downshift from 37°C to 10°C or exposure to chloramphenicol [14].

Mechanisms of bacterial cold shock response: basic concepts

The most prominent responses in *E. coli* and *B. subtilis* in response to cold shock is the induction of cold shock proteins along with the activation of certain systems such as desaturases, proteins chaperones, and trehalose-synthesizing machineries to shield the cells [15]. Indeed, the most well-studied cold-shock response mechanism underlies the discovery of the cold-shock proteins A (CspA) family members which are known to be induced at low temperature [19]. Other cold-shock proteins include Caps (cold acclimation proteins), CspB, CspC and CspE which may interestingly impart the motility trait and the capacity of biofilm formation under stress [16,17,19,20]. Cold shock proteins perform vital functions, such as mRNA masking, coupling of transcription to translation and developmental timing and regulation, which aids in survival of microbes in cold stress [21].

The roles of Rpo S protein (encoded by the *rpo* S gene) and guanosine 5' triphosphate-3'diphosphate, ppp Gpp and guanosine 5' diphosphate-3'diphosphate, ppGpp (collectively abbreviated as (p)pp Gpp) are also significant the cold shock stress management within bacteria [22]. Under cold shock, the secondary structures of RNA surprisingly stabilize, which presumably slows down (i) the transcription elongation and (ii) the ribosomal movement on RNA. CspA homologues are speculated to function as 'RNA chaperones' (CspA, CspB, CspG and CspI) since they can destabilize the secondary structures in RNA which in turn may facilitate transcription and translation [15].

Studies on cold shock on *Pseudomonas* strains

Studies on cold shock response within *Pseudomonas* strains have also been done; however that's not that much as in case of *E. coli* and *Bacillus* spp. The principal notification about *Pseudomonas* cold shock response involves the expression of the csp A gene encoding the major cold-shock protein Csp A (highly similar to that in *B. cereus*). The *csp* A gene has been detected in three Himalayan psychotropic *Pseudomonas* strains after downshifting the temperature from 28°C to 4°C [23]. Translational studies revealed the continuous overexpression of the Csp A protein at the temperatures as low as 4°C in *Pseudomonas* spp. The survival mechanism of *P. fluorescens* was studied whereby it was found that the bacterium could grow in the range of 30 to 4°C due to a probable constitutive expression of a cold resistant protein (CRP) [21]. In *P. putida*, the production of cold shock proteins (Csp S) and the Caps during the growth at 5°C was characterized earlier [24]. Panicker and colleagues conducted an interesting study on 18 Antarctic *Pseudomonas* isolates whereby the cold-shock domain (CSD)-encoding genes, cap B and cspA were analyzed; however, only the presence of cap B was noticed (expressed at 6°C) [25].

Possible wet lab experiments regarding the cold shock response in *Pseudomonas* spp: recommendations

A comparative observation of the growth curves of *Pseudomonas* spp. would be comprehensive to analyze the culturable, VBNC, and the dead cells at low temperatures ranging from 0°C to 8°C. Morphological study of the bacterial cells under cold shock would impart knowledge on the DNA changing pattern as well as the cellular changes [26]. Detection of the possible candidate genes responsible for the adaptation of the bacterial species at low temperatures using the polymerase chain reaction (PCR) and the Reverse Transcriptase (RT)-PCR of the *pnp* and *cap* B genes in Pseudomonas spp. would be very much fruitful to ponder the molecular basis of cold shock. Study of the expression of the *rpo* S gene (encoding the general stress sigma factor, σ^s), and the *rpo* E gene (encoding the σ^E, responsible for the VBNC cell lysis) under the cold shock condition would also be advantageous. Additionally, it would be also interesting to examine the accumulation of the aggregates in the cultures under cold shock in the proposed study. Moreover, since there are corresponding genes and homologues to *rpo* E gene (encoding σ^E, systems similar to the *E. coli* σ^E-dependent cell lysis) may exist in many microorganisms including in *Pseudomans* spp. Such system

may be crucial for cell turnover in stressed growth condition like at low temperature.

Conclusion

The knowledge on the *in vitro* modeling and simulations of clod shock response within the cells of *Pseudomonas* spp. would increment further molecular insights into the existing facts regarding the survival strategies of bacteria within the environment. The brief information as well as the recommendations about the wet experiments stated in the current review would aid the molecular biologists to understand the possible involvement of cold shock chaperons and to specifically identify the cold shock sigma factor (s). Finally, the possible fate of the VBNC cells of *Pseudomonas* spp. upon cold shock would be interesting to decipher the exact survival strategy of a bacterial population under a given condition.

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Conflict of Interest

None.

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