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Research Article

Cold Shock and Thawing Effect on the Growth of *Escherichia coli*

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Abstract

The different environmental condition is the most highly conserved on cold shock and heat shock features among all organism's cellular mechanisms. Cold shock and immediate thawing leads are kinds of cell stress that accumulation of partially and fully effected proteins that reflect external and eternal changes on cellular functions of all types of organisms. The objective of the examination was to determine the growth expression and physiological changes of *Escherichia coli* at cold temperatures after a certain time in our laboratory phenomena. The present study continued the investigation on *Escherichia coli* that influence of low temperature on growth behavior of coliform on Luria media. A slight retardation in the colony, colony count and cell morphology was noticed for coliform at 4°C after 48 hours and the 24 hours of incubations. Results confirm the potential to be significantly examined which can reveal new and vital associations.

Keywords: *Escherichia coli; Cold Shock; Thawing Effect; Growth Behavior*

Introduction

Different environmental condition such as nutrient and oxygen availability, osmotic stress which is influenced by temperature that is constantly adapted by micro-organisms. After exoneration of temperature, some important changes observed in cellular physiology of *Escherichia coli,* such as membrane fluidity has been diminished and the secondary structures of nucleic acids has been stabilized also, which is accountable for the depressed efficiency of RNA transcription, translation, and degradation.

Bacterial cell to prevent these hostile changes, mostly by the selective production of a specific set of proteins (cold-induced proteins, phospholipids) and the cold shock response has enabled it consequently. It increases in membrane permeability and a decrease in membrane fluidity. The Gram-negative cells membrane is composed of lipopolysaccharides (LPS), which is consist of a distal polysaccharide (O-antigen), and a core polysaccharide and lipid A. *Escherichia coli* (lipid A) comprise of two glucosamine with attached acyl chains (fatty acids). Laureate is the fatty acyl chain frequently observed in the cells growing at 37°C. At low temperatures, there is a decrease in laureate which is counterbalanced by the presence of palmitoleate [1]. The Emergence of palmitoleate increases membrane fluidity by the effect of low temperature because of palmitoleate being an unsaturated acid. Attaching palmitoleate to lipid upon temperature downshift that occurs by acyltransferase LpxP in *E. coli* which is induced by Cold temperature. Adaptation ability of membrane fluidity in *Bacillus subtilis* involves in rapid desaturation of fatty acids that subsit as phospholipids. It is the outcome of fetching of fatty acid desaturase (Des) which is adjusted by the sensor kinase DesK and the responsible as regulator DesR. Probable sensor of membrane fluidity was described by the transmembrane domain of DesK [2]. Decreasing membrane fluidity by lower temperature, which is aid the active state of the DesK kinase; DesK phosphorylates the transcriptional activator DesR which is fix with the promoter of the des gene and activates the synthesis of the enzyme D5-desaturase which is catalysis the introduction of a double bond into preexisting fatty acids tails of phospholipids inside the bacterial cell membrane [2,3]. Our prior studies transpired the genetic regulation and the impact of the temperature down-shift on the generation of oxidative stress response in *Escherichia coli* [4] and the physiological influence of the external and internal oxidative stress in different bacterial cells has been inquired [4-7].

In this study, we tried to make an evidence for temperature adaptation response of the coliform starter *E. coli* to a freezing condition. Protein synthesis is required for this adaptation prominently needs protein synthesis. Synthesized proteins are found in the class of chaperones protein. Furthermore, a chaperone gene is characterized, and its expression is studied after exposure to low temperatures.

Materials and Methods

Cultural condition of bacterial strain on culture medium

Laboratory stock cultures of *Escherichia coli* has been used in this experiment. Luria agar (LA) was used for the assay of bacterial culture [6]. EMB agar plates with bacterial culture was incubated at 37°C. After 24 hours incubation one loopful of bacterial culture was inoculated into 3 ml Luria broth (LB) which followed by 0 rpm (rotation per minute) at 37°C for 4 hours (pre-culture). Then store the Broth media at 4°C. Each experiment was done three times. Determining P value that is throughout the t-test beside measuring the Standard deviations was performed for statistical analysis of bacterial growth.

Microscopic analysis

Simple staining (Crystal Violet, Hucker's Solution) was applied for the observation of bacterial cell morphology and arrangements. An fractional part of 10 µl from each bacterial culture suspension was removed at 24 hours' intervals and the shape and organization of bacterial cells were observed under the light microscope at 1000× magnification.

Spread plate test

As described previously, each of the bacterial culture suspensions was 100 µl was dropped on to the LA plates, spread and dried off for 15 minutes, and finally, the plates were incubated at 37°C for 24 hours. The microscope was done at every 24 hours of growth from the LA plates.

Results

Colony-forming units (CFU) count of *Escherichia coli*

The total volume of the broth culture was 3 ml and the sample taken for spread was 100 µl. The countable colony of cultivable cell population due to cold stress according to the time. CFU count = bacterial colony number × dilation factor.

Time durations	Bacterial colony number	CFU count (approximately)
Day 2	> 401	1.2^{05}
Day 4	> 398	1.19^{05}
Day 6	> 403	1.21^{05}
Day 8	> 402	1.2^{05}
Day 10	> 410	1.23^{05}
Day 12	> 397	1.19^{05}
Day 14	> 400	1.2^{05}

Table 1: *Bacterial colony count and CFU result of cultivability and survival potential of E. coli upon low temperature treatment.*

Growth result of *E. coli* upon cold shock

There are no significant changes were observed in cell turbidity (Figure 1) as well as in CFU at 4°C temperature which is consider as low temperature in Luria broth media. But at growth retardation of bacteria observed up to 2 weeks of incubation.

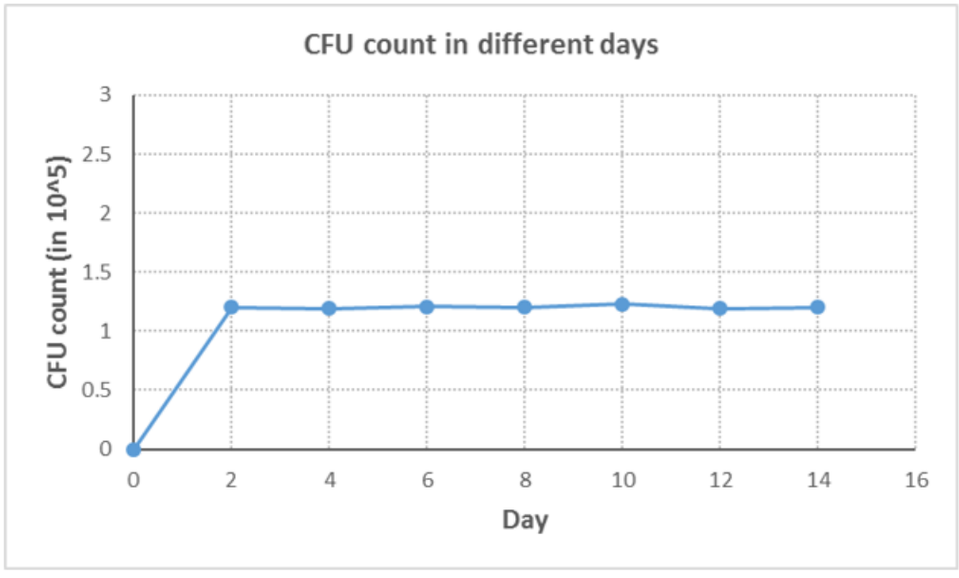

Figure 1: *Assessment of cell cultivability through the examination of growth of E. coli upon low temperature treatment. Bacterial cells were grown in Luria agar as described in materials and methods.*

Morphological changes of *E. coli*

In cohort with the previous findings of growth pattern no morphological change was documented for *E. coli* when they were subjected to grow at low temperature in LA (Figure 2) for 24 hours.

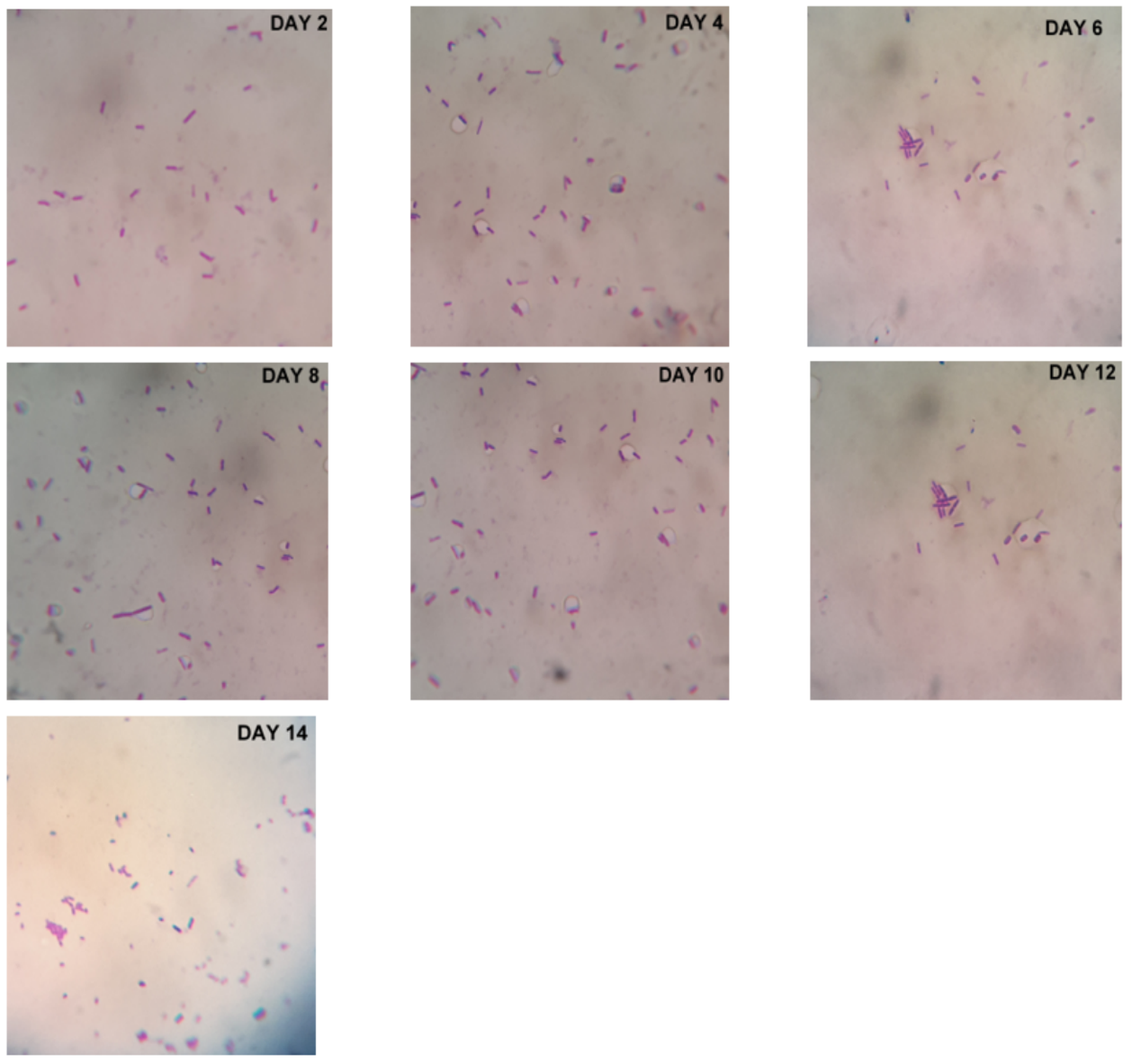

Figure 2: *Morphological examination of E. coli spp. upon low temperature treatment.*

Discussion

Analysis of the growth characteristics of the LAB has resulted in a grouping into mesophilic strains. Mesophilic organism *E. coli* strain used in this experiment, which has an optimal growth temperature of approximately 37°C. The minimal growth temperature of Escherichia coli was shown to be slightly lower than 10°C (exact minimal growth temperature 7.5°C), whereas the theoretical minimal growth temperature appeared to be 7.5°C. *E. coli* was able to recover from a cold shock treatment with a temperature drop of about 20°C within 48 hours to 2 weeks, indicating that this strain has no capacity to efficiently adapt to low temperatures. However, a cold shock from 37 to 4°C resulted in a growth block (Figure 1). There was no significant morphological change observed under the microscope after 48 hours but growth retardation of bacteria observed up to 2 weeks of incubation. The high thawing effect inhibits the growth of *E. coli* as well as low temperature. Coliform bacteria like *E. coli* can be inhibited by antimicrobial treatment but cold and heat shock treatment has natural aspects. The chaperone protein and genes of *E. coli* cannot express their genetic alleles to producing offspring and do not attempt to protein fold by expressing chaperones and inhibit the growth of mesophilic organism such as *E. coli*. The heat shock

response in bacteria is a protective mechanism to cope with heat-induced damage to proteins by synthesizing a specific set of proteins known as heat shock proteins (HSPs) [8]. The alternative sigma factor σ32 mediates the heat shock response. Under stress conditions, an elevated environmental temperature causes a transient increase in σ32 transcription and transient stabilization of σ32 protein levels, which is usually unstable. The σ32 directs transcription of RNA polymerase (RNAP) from the heat shock promoters and, thus, results in the induction of HSPs. Most HSPs behave as molecular chaperones that function to bind to and stabilize non- native polypeptides that are generated during protein synthesis or by heat denaturation of existing proteins, modulate protein folding pathways to prevent miss-folding or aggregation of proteins and promote protein refolding and proper assembly [9]. A number of molecular chaperones have been identified in *E. coli*, including DnaK (HSP70), DnaJ, GrpE, GroEL (HSP60), and GroES and most of them are heat inducible [10,11]. In addition, some HSPs are ATP-dependent proteases and play major roles in digesting irreversibly heat-damaged polypeptides for removal and assist in nucleic acid synthesis, cell division, and motility under normal and stress conditions [12]. Some of the HSPs are essential for growth at a higher temperature and are involved in various cellular processes such as proteolysis, cell wall synthesis, cell division, phase growth, and plasmid DNA replication [10,13,14]. Sub lethal heat stress (heat shock) or prior exposure to low heat may render *E. coli* O157:H7 microorganisms more resistant to subsequent heat treatment, which would otherwise be lethal [15,16]. HSPs play a crucial role in this stress response [17-78].

Conclusion

A multifaceted response on the wide variety of stresses has to mounted by *Escherichia coli* which also encountered by these type of organisms. The adaptive response for the bacterial survive varies on the variations of the temperature range. They have shown an extensive variety of survival strategies and temperature-related changes to the cold stress. There are no morphological characteristics changes and other property of variations like shrinking or elongating were not observed. This is the significant invention in the strategies and phenomena of this study and it occurs in microorganism's resistance mechanisms against cold stress. This experiment has been fueled through an important information in the related field from previous researches which has been worked with identifying the effect and mechanism of different stresses including cold shock and heat shock on certain kind of bacteria such as *Escherichia coli.*

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Author contributions

A.S., S.I. and M.F.H. performed experiments. A.S. and M.F.H. performed the microscopic analysis. A.S. and T.B.E. performed the statistical analysis. A.S., S.I. and T.B.E. conceived the study and designed the experimental procedures. T.B.E. supervised the study. A.S. and T.B.E. wrote the manuscript. All authors read and approved the manuscript.

Competing Interests

The authors declare that they have no competing interests.

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