

Original Research Article

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Studies on the Protein Profiles of Heat and Cold shocked Bacteria

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ABSTRACT

The purpose of this study was to detect the changes in the protein profiles for bacteria which were subjected to heat and cold- shock compared to the control. For heat shocked test, all bacteria viz., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus* were inoculated into nutrient broth tubes and incubated at 37°C for 24 hours. Then cultures were treated in a water bath at 100°C for 5 mins, 90°C for 10 mins, 80°C for 15 mins, 70°C for 20 mins and 60°C for 25 min. After that the protein profiles and their concentrations were estimated to detect the differences compared to the control. For cold shocked test the cultures were treated at -32°C, -15°C and 0°C for 30 min. After that protein profiles and concentrations were estimated to detect the differences compared to the control. From chromatogram of *Staphylococcus aureus* it is evident that apparent changes were noticeable upon raising of temperatures from -32°C to 100°C. The protein bands were focused between 75 kDa to 11 kDa. In case of *Staphylococcus epidermidis*, unlike *Staphylococcus aureus*, there was no apparent electrophoretic difference in the protein expression from low to high temperature .On the other hand, concerning protein estimation, there was an increase in protein by more than 140% as the temperature rises from -32°C to 100°C. Protein bands were focused between 75kDa to 11 kDa. Although there was an apparent variation in the protein composition of *Bacillus cereus* with gradual increase of temperature, there were no clear electrophoretic bands discrepancies from -32°C to 100°C.

Keywords

Gel electrophoresis,
Chromatogram,
Protein profiles,
Estimation, Heat
and cold-shock

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Introduction

Heat-shock proteins are named according to their molecular weight. For example, Hsp60, Hsp70 and Hsp80 (the most widely- studied HSPs) refer to families of heat shock proteins on the order of 60, 70, and 80 kilodaltons in size, respectively (Li and Srivastava, 2004).

The small 8-kilodalton protein ubiquitin, which marks proteins for degradation, also has features of a heat shock protein (Raboy, 1991).

Heat shock proteins are also believed to play a role in the presentation of pieces of proteins

(or peptides) on the cell surface to help the immune system recognize diseased cells (Tsan and Gao, 2009).

Electrophoresis is a technique used to separate and sometimes purify macromolecules - especially proteins and nucleic acids - that differ in size, charge or conformation. Low temperature can influence the response of a microorganism either directly or indirectly. Direct effects include decreased growth rate, enzyme activities, alteration of cell composition and differential nutritional requirements. Indirect effects are usually observed on the solubility of solute molecules, diffusion of nutrients, osmotic effects on membranes and cell density (Shekhar, 2011).

A bacterial cell has to react immediately when exposed to unfavorable external condition, through such responses as entering the stationary or sporulation phase. Cold shock occurs when bacteria are exposed to sudden down shifts in temperature (Wilson and Nierhaus, 2004).

These so-called 'cold shock' proteins are thought to help the cell to survive in temperatures lower than optimum growth temperature, by contrast with heat shock proteins, which help the cell to survive in temperatures greater than the optimum, possibly by condensation of the chromosome and organization of the prokaryotic nucleoid (Obokata, 1991).

Taken all together, it is likely that expression of all four csp genes is regulated essentially in the same manner. It should be mentioned, however, that the optimal temperature ranges for the induction of these genes are different (Etchegaray *et al.*, 1996; Wang *et al.*, 1999).

Materials and Methods

Electrophoresis is a technique used to

separate and sometimes purify macromolecules - especially proteins and nucleic acids - that differ in size, charge or conformation. The buffers for SDS were prepared according to Schagger and Jagow (1987). Acrylamide/Bis solution 29:1 is also suitable for preparation of a gel.

Bacterial protein extraction procedure for all of isolates was applied by taking 300ul of sample loading buffer solution was added to the resuspended cells and pipetted up and down to lyse the cells.

The protein extraction for estimation was done by adding PBS to harvest cells. Cells were treated with the Sonicator for 5 minutes for all isolates except *S.aureus* which was treated for 10 minutes.

The value of molar absorption coefficient was calculated using the equation ($Y = mx + b$). Where Y =absorbance at 595nm and X =protein concentration and b =protein concentration per mcg/ml (micrograms per microliters). The reading of protein concentrations was duplicated to avoid the error and to get the accurate means from the repetitions of the reading samples.

Results and Discussion

Sodium dodecyl sulfate-polyacrylamide gel-electrophoresis (SDS- PAGE) appears in the same results for the running of the protein into the gel electrophoresis, however, the variation that was not detect through protein profiles was clear in the estimations of protein. Upon quantifying the amount of protein of *Staphylococcus aureus* obtained upon lysis of cell, there was clear increase in protein expression (mg/ml) from -32°C to 0°C. After shocking at 0°C the content of protein remained almost unchanged. The electrophoretic pattern of proteins of *S.aureus* isolates, stored at different temperatures is shown in figure (1A).The estimation of the

protein concentrations is shown in figure(1B).

The electrophoretic pattern of proteins of *S.epidermidis* isolates, stored at different temperatures is shown in figure (2A). The estimation of the protein concentrations is shown in figure (2B).

The electrophoretic pattern of proteins of *B.cereus* isolates, stored at different temperatures is shown in figure(3A).The estimation of the protein concentrations is shown in figure(3B).

Pathogenic bacteria can develop systems that assist them to survive and adapt to environmental stresses such as heat and cold in a variety of ways. These responses entail the production of protective proteins, some of which offer protection to more than one type of stress. *Staphylococcus aureus* are known to produce these cold shock proteins, although the ability to produce and intensity of production may vary from strain to strain (Cordwell *et al.*, 2002).

Figure.(1A) Electrophoretic patterns of proteins of *S.aureus* isolate which was stored at different temperatures show similar results. Molecular mass markers are indicated. Equal quantities of protein were loaded

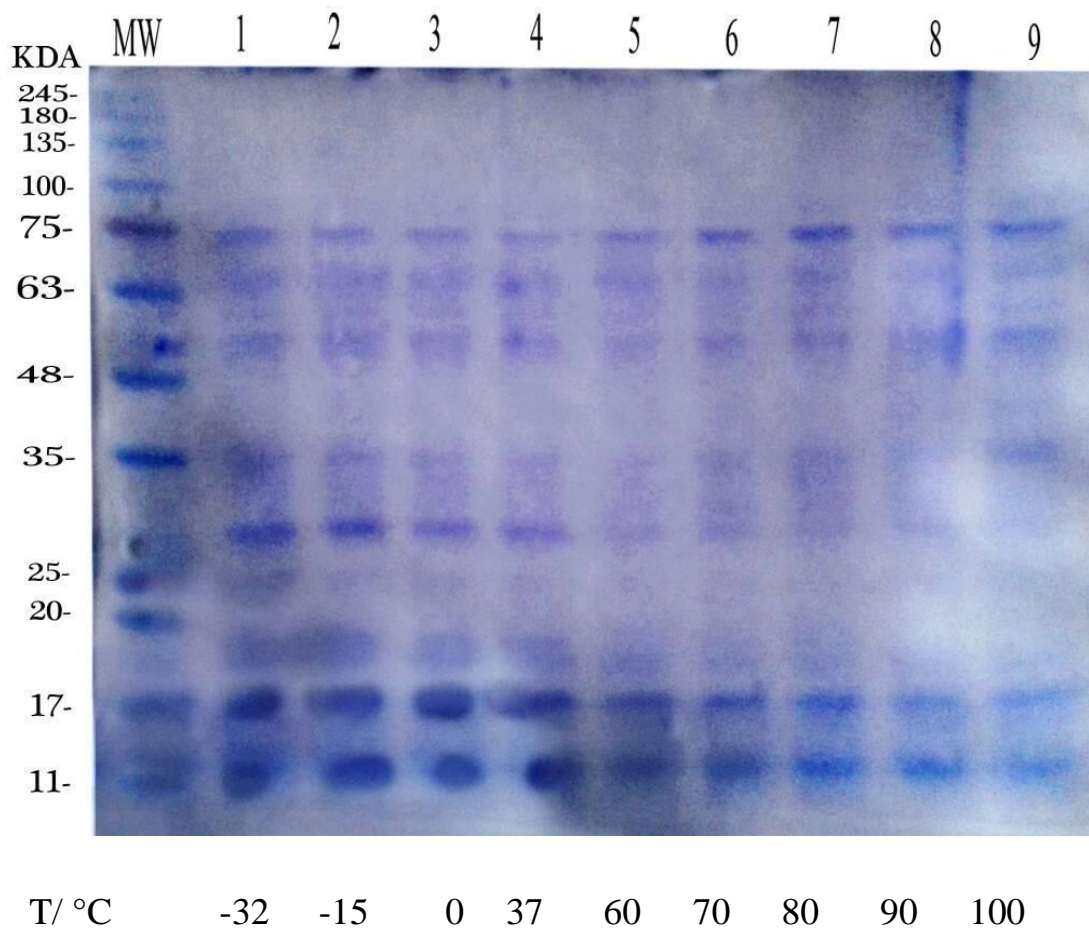


Figure.(1B) Estimation of protein concentration of *S.aureus* which was stored at different temperatures (-32°C, 100°C) using standard graph of BSA

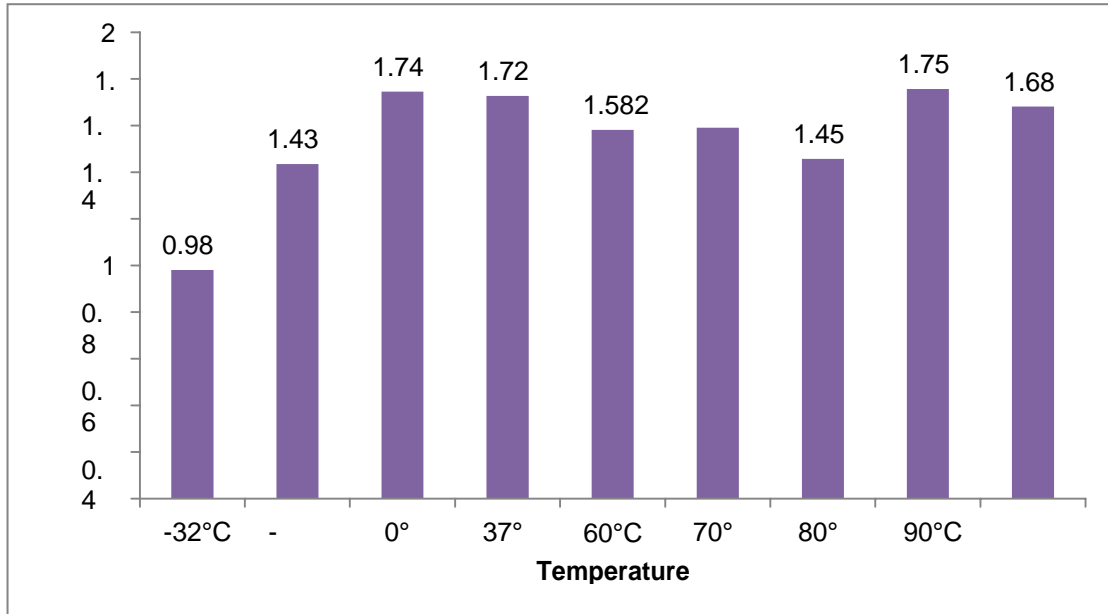


Figure.(2A) Electrophoretic patterns of proteins of *S. epidermidis* isolate which was stored at different temperatures show similar results. Molecular mass markers are indicated. Equal quantities of protein were loaded

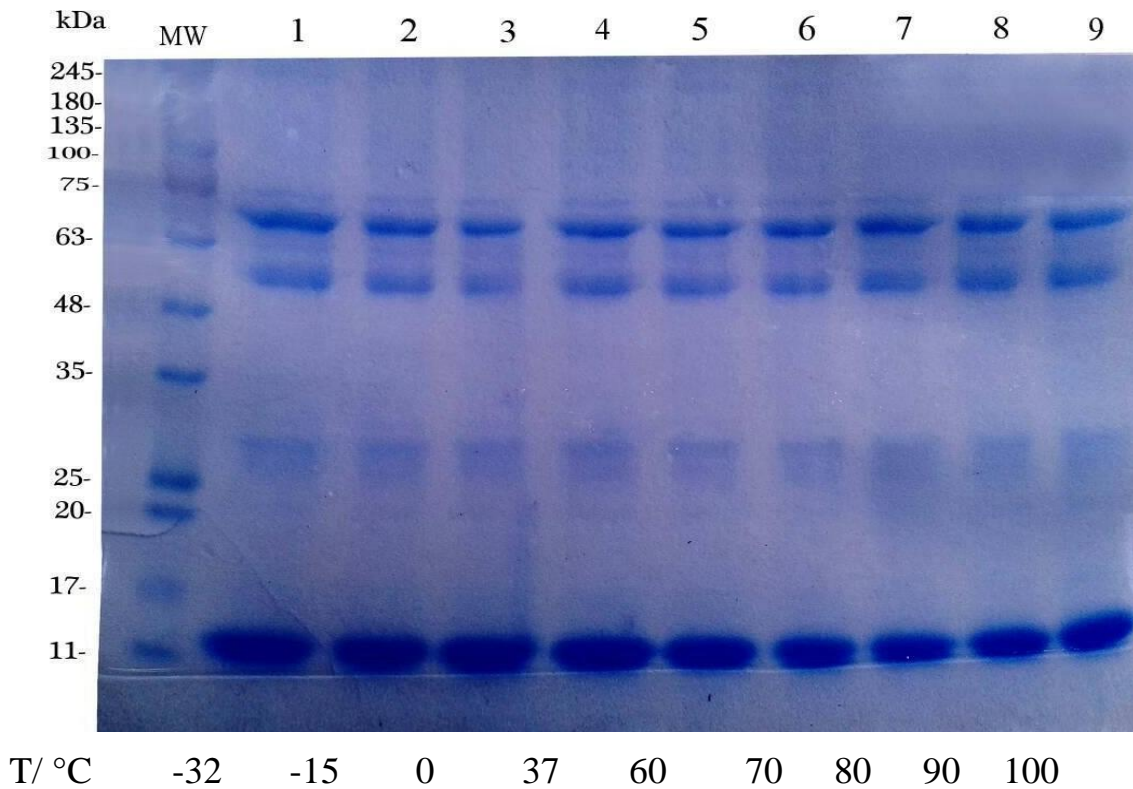


Figure.(2B) Estimation of protein concentration of *S. epidermidis* which was stored at different temperatures (-32°C, 100°C) using standard graph of BSA

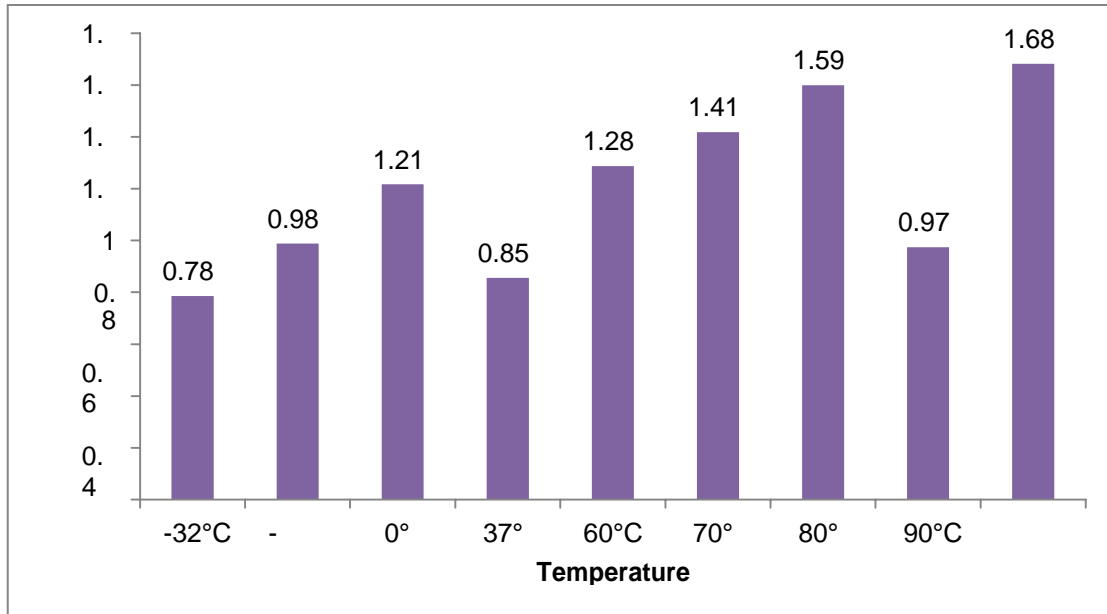


Figure.(3A) Electrophoretic patterns of protein of *B.cereus* isolate which was stored at different temperatures show similar results. Molecular mass markers are indicated. Equal quantities of protein were loaded

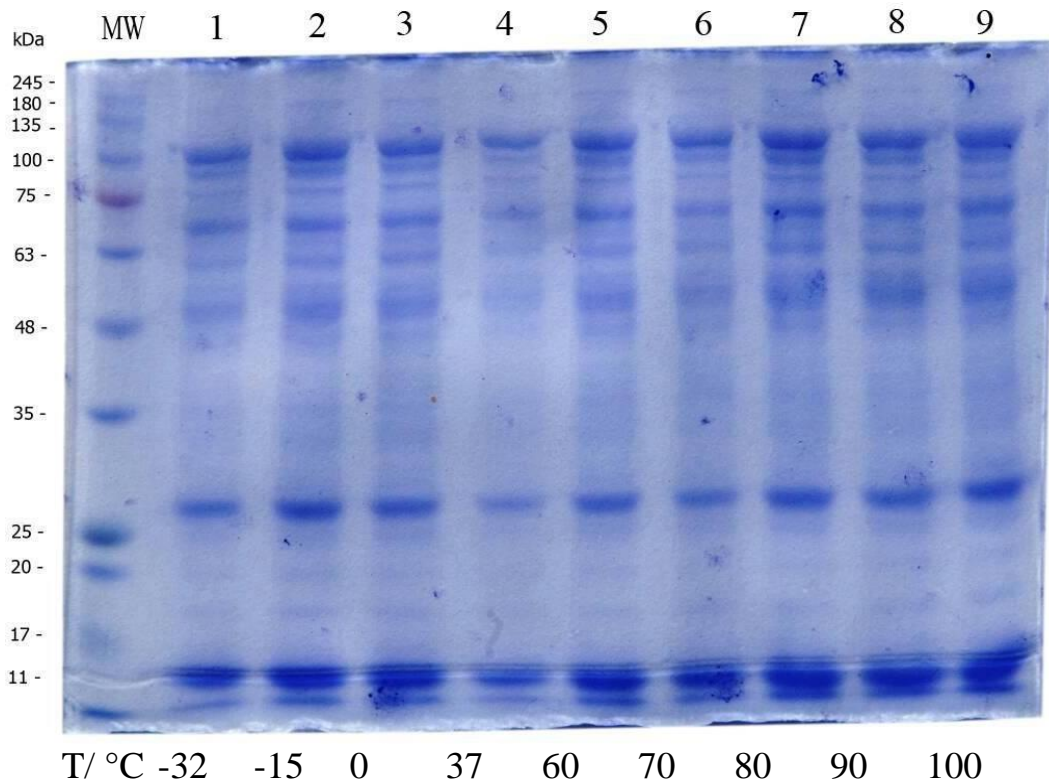
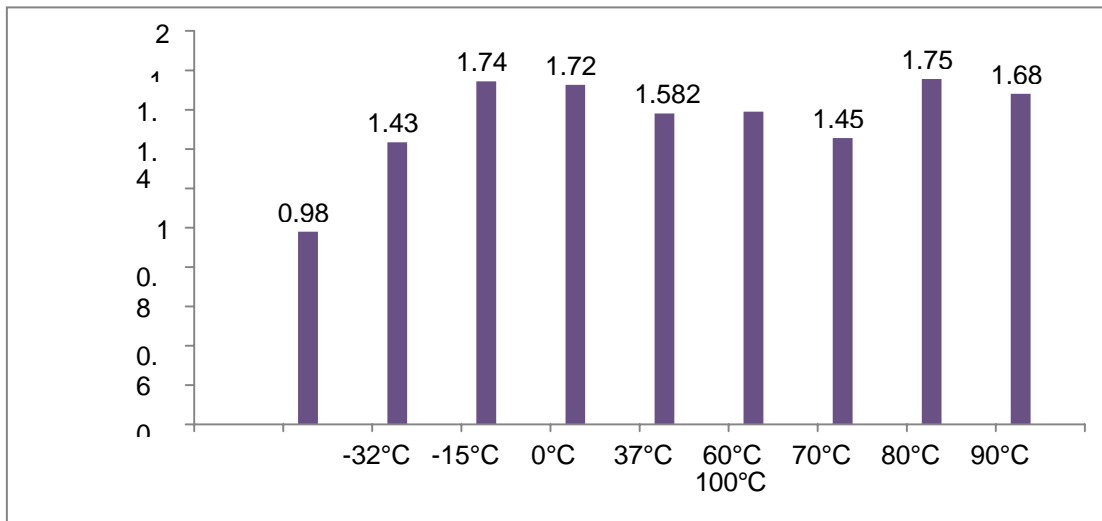


Figure. (3B) Estimation of protein concentration of *B.cereus* which was stored at different temperatures (-32°C, 100°C) using standard graph of BSA.



Sheridan and McDowell (1998) reported that chilling did not enhance the resistance of *S.aureus* to subsequent thermal inactivation. While the currently recommended mild cooking conditions of 70°C for 12 min would still achieve a 5 log reduction in *S.aureus*, which is generally accepted to be the target reduction for food-borne pathogenic bacteria (Anonymous, 2001).

Such treatment would not allow for the error which is normally incorporated into such recommendations. Thus, pasteurization at a time- temperatures combination of anything less than 70°C for 20 min might result in the survival of *S.aureus* allowing for subsequent growth and toxin production if the product was not chilled after cooking. Re-heating would have little effect on the heat stable (Fung *et al.*, 1973).

From chromatogram of *Staphylococcus aureus* in figure (1A), it is evident that apparent change is noticeable upon raising of temperatures from -32°C to 100°C. Though the difference appears closely associated with pattern of protein expression rather than presence or absence of the protein. The

protein band appeared at molecular weight 70 kDa seems to be up regulated; whereas the one above 25kDa and below 35 kDa (indicated with arrow) is down regulated as the temperature rises up. The protein bands were focused between 75 kDa to 11kDa. In case of *Staphylococcus epidermidis* (figure, 2B) and unlike *Staphylococcus aureus* there was no apparent electrophoretic difference in the protein expression from low to high temperature (figure,2A).

On the other hand concerning protein estimation, there was an increase in protein content by more than 140% as the temperature rises from - 32°C to 100°C (figure 2B). Protein bands were focused between 75 kDa to 11 kDa. Though there was apparent variation in the protein composition of *Bacillus cereus* with gradual increase in temperature there were no clear electrophoretic bands discrepancies from - 32°C to 100°C as shown in figure (3A).

Cooking temperatures less than or equal to 100 °C (212 °F) allow some *B.cereus* spores to survive (Roberts *et al.*, 1996). This problem is compounded when food is

improperly refrigerated, allowing the endospores to germinate (McKillip, 2000).

In conclusion, though Sodium Dodecyl Sulphate Poly acrylamide gel electrophoresis (SDS PAGE) showed similar results in protein profiles pattern under both cold and heat shocked bacteria conditions, apparent variations in protein contents are obvious.

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