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STUDIES ON THE PROFILE OF HEAT SHOCK PROTEINS IN SELECTED HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Heat shock proteins are evolutionarily highly conserved polypeptides that are produced under a variety of stress conditions to preserve cellular functions. In the present study, The bacterial species *Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris, Staphylococcus aureus, Klebsiella pneumoniae* were grown in their respective selective medium for identification of the species. The identified species were grown in nutrient broth for 48 hours at 37°C in a rotary shaker. About 5 ml of each culture was transferred to new and sterile tubes and were incubated at 50°C for an hour (Heat shock).Then both the cell types ie., heat shocked cells and normal cell suspensions were centrifuged at 10, 000 rpm for 15 minutes. The supernatant was loaded in the wells of the SDS-PAGE gel. The protein bands were observed and heat shock proteins were identified by comparing the protein profile of the normal as well as heat shocked cells. The molecular mass of the suspected heat shock proteins were determined by comparing with the molecular mass markers. All the bacterial cells showed an increase in the total cellular protein content after heat shock.

KEYWORDS: *Heat shock proteins, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris, Staphylococcus aureus, Klebsiella pneumonia, molecular mass markers.*

INTRODUCTION

Heat shock proteins are present in cells under normal conditions, but are expressed at high levels when exposed to a sudden temperature hump or other stress. The induction of the heat shock response in Escherichia coli involves the synthesis of atleast 20 proteins. Heat shock proteins (HSPs), also called stress proteins, are a group of proteins that are present in all cells

in all life forms. They are induced when a cell undergoes various types of environmental stresses like heat, cold and oxygen deprivation.

HSP are involved in the assembly and disassembly of multimeric protein structures, the translocation of proteins across membranes and the secretion and degradation of Proteins. HSP also stimulates protein glycosylation **(**Lilia compadre *et al*., 1996). The heat shock response is a defense reaction activated by proteotoxic damage induced by physiological or environmental stress. Cells respond to the proteotoxic damage by elevated expression of heat shock proteins (Hsps) that function as molecular chaperones and maintain the vital homeostasis of protein folds.

The heat shock response is a defense reaction activated by proteotoxic damage induced by physiological or environmental stress. Cells respond to the proteotoxic damage by elevated expression of heat shock proteins (Hsps) that function as molecular chaperones and maintain the vital homeostasis of protein folds Heat shock factors (HSFs) are the main transcriptional regulators of the stress-induced expression of *hsp* genes (Johanna., 2006). Heat shock proteins (Hsps) have become useful models for studying transcriptional regulation, the stress response and molecular evolution. (Zhongliang Wang., 2008).

Extracellular localized HSP have been found to play key roles in the induction of a cellular immune response. Either they act as carrier molecules for immunogenic peptides that are presented on Antigen Presenting Cells (APC) to cytotoxic T-cells or they themselves act as activatory molecules for the innate immune system (Gabriele Multhoff., 2009).

Organisms respond to elevated temperatures by rapidly inducing HSPs. The regulation of this response is mediated at the transcriptional level by a transcriptional level by a transcription factor called heat shock factor(HSF), which binds to the regulatory shock el ements that are present upstream of all heat shock genes. Thus, HSF responds to the heat shock signal by binding to DNA and regulation of target gene transcriptional competence.

In the present study, an attempt was made to isolate and characterize the heat shock proteins from selected human pathogenic bacteria, based on their approximate molecular mass. The bacterial species were isolated to characterize heat shock proteins are *Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris, Staphylococcus aureus and Klebsiella pneumonia*. Giving heat shock to the bacteria at 50° C. Lysis of the bacterial cells, Estimation

of total cell protein content in normal and the cells which are subjected to the heat shock, Electrophoretic separation of the bacterial lysate by SDS-PAGE and determination of the molecular mass of the individual protein bands by comparing with that of the standard molecular mass markers and Identification of possible heat shock proteins by determination of their relative molecular mass.

MATERIALS AND METHODS

In this present study, The following human pathogenic bacteria were isolated to characterize heat shock proteins. In this study the following human pathogenic bacteria were isolated to characterize heat shock proteins. *Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris, and Klebsiella pneumonia* based on standard cultural and biochemical techniques.These Bacteria were inoculated in their respective selective medium and incubated at 37°C for 24-48 hours. The colonies were checked for purity.

Growing The Cultures in Liquid Medium

Then the representative colonies were inoculated in 5 different Erlen-Meyer flasks containing about 50 ml of nutrient broth in each and incubated at 37°C for 24-48 hours till the desired cell density is reached, in a rotary shaker.

Heat Shock Treatment

Then about 5 ml of each culture was transferred to 5 separate sterile test tubes and were incubated at 50°C for one hour.

Centrifugation

The cell mass in each tube was harvested by centrifuging the contents of the tube at 10, 000 rpm for 15 minutes and each tube was labeled.

Then about 5 ml of each culture from the rotary shaker which were incubated at 37°C were transferred to 5 sterile tubes and centrifuged at 10, 000 rpm for 10 minutes after labeling the tubes.

Cell Lysis

The supernatant was poured off. The resulting bacterial cell pellets were suspended in 300 micro litre of lysis buffer, consisting of 62.5 mm Tris-HCL(pH 6.8), 2% sodium dodecyl sulphate, 10% Glycerol, 5% 2-mercaptoethanol and 0.001% bromophenol blue. This suspension was agitated vigorously after the addition of glass beads in a vortex mixer. The

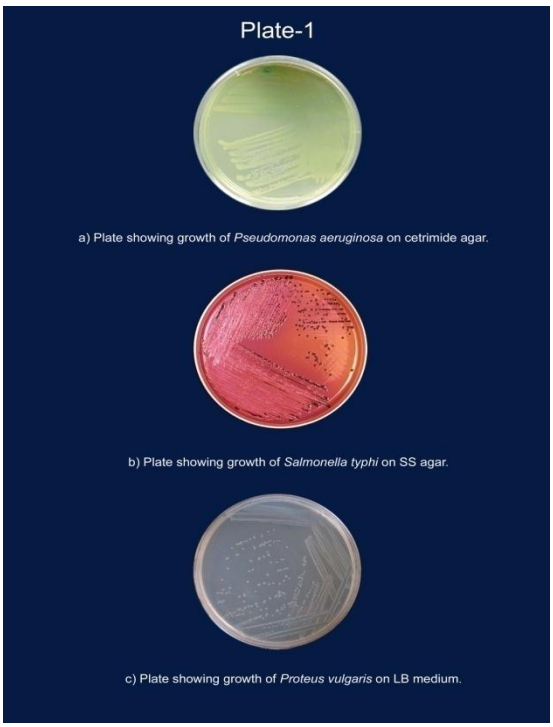
resulting cell lysate was subjected to heating at 100°C for 5 minutes. Again the cell lysate was centrifuged at 10, 000 rpm for 15 minutes and the supernatant was loaded in the wells and electrophoresed using SDS-PAGE.

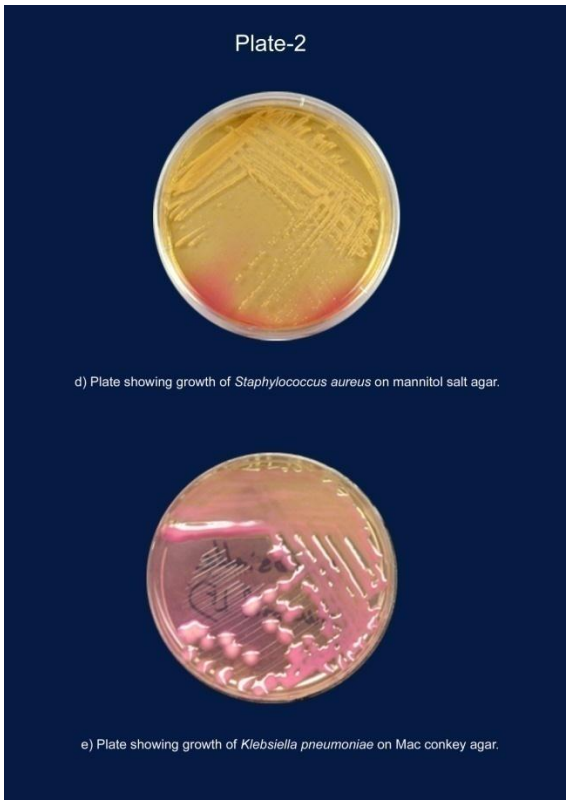
Protein Estimation By Lowry's Method (Oliver H. Lowry,1940)

Then protein estimation was carried out by Lowry's method (Oliver H. Lowry,1940).1.5 ml of culture from each sample (totally 10) was taken in 10 sterile tubes and 1.0 ml of 1.25 N sodium hydroxide solution was added to each tube and all the tubes were kept in a boiling water bath for ten minutes. The absorbance was read at 660 nm spectrophotometrically. The protein content of each species after heat shock were compared with that of those prior to the heat shock.

RESULTS

In this study, it was attempted to induce the synthesis of heat shock proteins in selected human pathogenic bacterial species Bacteria were streaked in their respective selective medium for purification and identification. *Pseudomonas aeruginosa* were isolated on Cetrimide agar(*Plate*-1,a).*Salmonella typhi* were isolated on SS agar (*Plate*-1,b).*Proteus vulgaris* were isolated on LB agar (*Plate*-1,c).*Staphylococcus aureus* were isolated on Mannitol salt agar (*Plate*-2,d).*Klebsiella pneumoniae* were isolated on Mac Conkey agar (*Plate*-2,e). and the respective colonies were inoculated in Nutrient broth and were incubated at 37°C for 48 hours in a rotary shaker.





The protein content of each bacterial species before and after heat shock was estimated by Lowry's method. The results in terms of optical density for each species before and after heat shock were recorded in *Table:1*.

Table:1

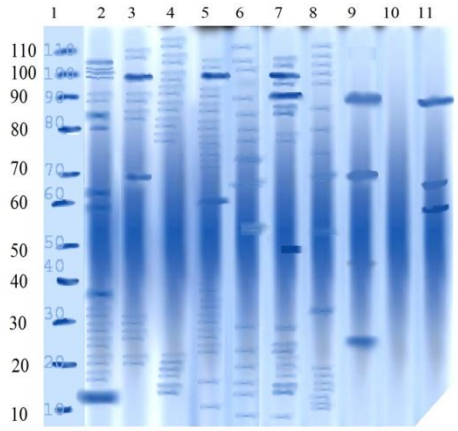
The

Organism	Protein Content(IN O. D)	
	Before Heat Shock	After Heat Shock
Proteus vulgaris	0.46	0.48
Staphylococcus aureus	0. 41	0. 43
Salmonella typhi	0. 39	0. 44
Klebsiella pneumoniae	0. 45	0. 61
Pseudomonas aeruginosa	0. 43	0. 46

protein bands that were seen in those lanes which were loaded with the lysates of the bacterial cells which were subjected to heat shock were supposed to be heat shock proteins. As there were no confirmatory tests, it was concluded that those bands which were not present in the lanes loaded with the lysates of Bacterial cells which were grown at 37°C but which were present in those lanes loaded with the lysates of Bacterial cells which were subjected to heat shock and falling in the molecular weight ranges of 100 Kda, 90 Kda, 70 Kda and 60 Kda were possibly identified as Heat Shock Proteins. Lane 11 was loaded with the lysate of the

heat shocked cells of *Klebsiella pneumoniae* and it showed protein bands of molecular mass of 100Kda, 70 Kda and 60 Kda.(Shown in Plate:3)

Plate-3



Lane 1	Marker
Lane 2	PVU 37
Lane 3	PVU 50
Lane 4	PS37
Lane 5	PS50
Lane 6	STY 37
Lane 7	STY 50
Lane 8	SAU 37
Lane 9	SAU 50
Lane 10	KL37
Lane 11	KL50

f) SDS-PAGE gel showing bands of heat shock proteins.

DISCUSSION

All the bacterial species which were used in this study, showed an increased synthesis of proteins when incubated at 50°C for an hour than those which were incubated at 37°C. It showed that the organisms respond to the heat shock by synthesizing some novel protein families and synthesized them in large amounts, so that they could protect the cell from destruction by increased temperature.

The culture suspension was centrifuged to obtain the cell pellet and the cell pellet was lysed by suspending in 1. 25 N NaOH solution and it was kept in a boiling water bath for 10 minutes. Protein estimation was done by Lowry's Method and absorbance was read at 660 nm. All the bacterial cells showed an increase in the total cellular protein content after heat

shock. It was observed that all the species of bacteria expressed higher levels of protein after heat shock. Heat shock response in *Escherichia coli* was monitored using an optical biosensor. A surface plasmon resonance (SPR) method for monitoring the concentration of the chaperone DnaK and its relation to physiological stress response in a recombinant Escherichia coli strain subjected to heat shock is described (Carl-Fredrik Mandenius., 2003). The molecular mass of the suspected heat shock proteins were determined by comparing with the molecular mass markers loaded in the lane 1.The suspected heat shock protein were of the molecular mass ranges of 60 Kda, 70 Kda, 90 Kda and 100 Kda. According to the molecular mass, Hsps are classified into several families, such as Hsp90 (85–90 kDa), Hsp70 (68–73 kDa), Hsp60, Hsp47 and low molecular mass Hsps (16–24 kDa**) (Zhongliang Wang., 2008).**

CONCLUSION

Heat shock proteins (Hsps) are the most abundant and ubiquitous soluble intracellular proteins which are phylogenetically conserved in all organisms .From the results of the above experiment, it was inferred that heat shock in some way or the other induces protein expression to a maximum, and also it might have induced some special protein species which would have protected the cell and helped it to survive the heat shock (i. e) the increase in temperature.

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