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THERMOTOLERANCE OF *Staphylococcus aureus* AFTER SUBLETHAL HEAT SHOCK

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ABSTRACT

The effect of prior heat shock on the thermotolerance of *Staphylococcus aureus* in broth culture and induction of heat shock proteins was investigated. *S. aureus* cell wall was, also, examined using Scanning Electron Microscope (SEM). Specific bacteria were grown at 37°C the optimum temperature (control), sublethally heated at 47, 52, 57 and 60°C for various times, and heat treated at 68°C were done. The estimated average of the D_{68}-values for the control was 4.83 min while for heat shocked treatments ranged from 5.43 to 10.23 min. The current results, also, indicated that 8 - 16 new bands were induced by applying heat shock treatments with molecular weights ranged from 9 to 135 KDa. The induced heat shock proteins further confirmed the increased the thermotolerance. Moreover, selected heat shock treatments caused severe destruction in cell wall (i.e., rupture, irregular and leakage of cell contents), while heat shocked cells after incubating at 37°C for overnight in enriched medium became similar to that of the one's normal. The enhanced heat resistance of *S. aureus* should be thought-about in cause of planning effective thermal processes to confirm the microbiological food safety.

Keywords: Thermotolerance**,** *Staphylococcus aureus,* Heat shock protein, Electron microscopy

INTRODUCTION

Bacterial cells are additional seemingly killed than stressed or injured throughout traditional food processing (e.g., sterilization and retorting). The heat process represents a gentle stress evoked adaptational response in bacterium. Adaptation of salmonella for acid stress raised survival of such microorganism in cheese. While, *L. monocytogenes* was heat shocked at 48°C for 120 min, exhibited adapted cells were enhanced tolerance to heat in sausage batter **(Farber and Brown 1990).** Acid adaptation enlarged survival of *L. monocytogenes* in acid foods like yoghurt; fruit juices and sauce **(Gahan, 1996).**

Certain process conditions give rise to stress adaptation and affects various foods safety. For example, acidity that developed through presence and fermentation of salt in soured sausage might induce an acid adaptive and diffusion shock response for pathogenic bacterium and should resist smoking treatment or heating or persist throughout product storage. Similarly, milk bacterium that heated at sub-pasteurization temperatures (e.g., for creating some types of cheese) might affected by gentle heat shock (heat stress). Such bacterium might become resistant to severe processing (e.g., preparation to the processed cheese). By increasing the utilization of different process technologies (as novel, non-thermal, or rising technologies) is arousing curiosity against the potential stress adaptation of food borne pathogens.

S. aureus have the ability to grow and survive at low water activity foods (a_w = 0.85) and caused large outbreaks from dry foods like powdered skim milk, and also when improper handling or temperature abuse occurs after processing **(Asao et al 2003).** The thermal resistance for species has been place into proof by some authors **(Kennedy et al 2005)** who discovered that *S. aureus* had larger D-value than *Listeria monocytogenes*.

The microorganisms' heat tolerance varies wide counting on factors that act before, through-

out or after process. One among these factors was exposure of bacteria to previous heat shock, i.e. high temperature in environmental before actual heat treatment. As compared to really products, a broth culture as heating medium is wide accustomed verify the heat resistance of pathogens **(Juneja et al 2001),** since it provides quick and uniform heating to realize isothermal conditions and should represent their intrinsic heat resistance characteristics in real food **(Leguerinel et al 2007).**

The exposure of bacterial cells to a heat shock provokes a rise in their heat tolerance which will have necessary practical consequences **(McMahon et al 2000 and Hassani et al 2006 and 2007).** Heat shock, plays as triggers, a physiological response results in synthesis of a specific proteins referred to as heat-shock proteins HSPs **(Schlesinger, 1990).** Synthesis of HSPs sometimes happen within 5 to 60 min after heat shock and descends with the onset of normal protein synthesis 60 to 90 min once come back to normal temperatures **(Watson, 1990).**

HSPs increase the potential of bacterium to resist severe future stresses and enhance the survival of pathogens throughout exposure to high temperatures. **Schlesinger (1990)** Stated that the role of HSPs in thermotolerance acts as chaperones to get rid of denatured proteins. Chaperones represent 15 to 20% of the overall cellular protein in response to elevate temperatures **(Arsene et al 2000).** The primary function of classic chaperones, *E. coli* DnaK (HSP 70), DnaJ, Grpe, GroEL (HSP 60) and GroES is to bind to polypeptides and modulate folding pathways to stop protein aggregation **(Georgopoulos and Welch, 1993),** additionally, as play, a vital role in protein folding, repair and turnover below stress and normal conditions **(Rosen and Ron, 2002; Schumann, 2007).**

The aim of this study is to: examine the effect of exposure *S. aureus* to heat shock at different times and temperatures, determine viability and heat resistance of microbial cells, investigate microbial cell wall using Electron Microscopy technique as well as to detect the presence of induced heat shock proteins by using polyacrylamide gel electrophoresis.

MATERIAL AND METHOD

1. Bacterial strain and bacterial conditions

S. aureus (ATCC 25923) was used. The strain was obtained from TCS Biosciences Company (Botolph Claydon, Buckingham, MK18 2LR, United Kingdom). Tested culture was grown in Baird Parker (Oxoid, England) at (37°C/24h), subcultured every month and kept at 4°C.

2. Preparation of cell suspension

Cultures were grown in buffered peptone water to exponential phase at 37°C/24hr, then harvested by centrifugation at 10000 rpm for 10min, washed twice with distilled water and resuspended in fresh buffered peptone water (LABM, United Kingdom) at a cell concentration of about 10^9/ml.

3. Sublethal heat shock treatment

Ten ml of bacterial suspension were subjected to heat shock for selected time periods i.e. 5, 15, 30 and 60 min at preselected temperatures between 47°C and 60°C with 5°C interval by immersion in a waterbath (M43 S.N.130805-42, Korea). Cultures were cooled in ice water prior testing for their tolerance to a high temperature at 68°C. A 1ml sample of heat shocked cells was transferred into 9 ml of preheated buffered peptone water and heated to 68°C for 15 min in test tube that was submerged in preheated water bath. Samples were withdrawn every 3min and immediately cooled in ice water. Control sample (non-heat shocked cells) was heated at 68°C for 15 min. Three replicates of each experiment were performed under the same conditions **(Li et al 2005).**

4. Determination of viability

Cell viability was determined by **Tosun and Gönül (2005).** Serial decimal dilutions in 0.1% buffered peptone water were prepared. The viable population of cultures was determined by plating 0.1 ml of the serially diluted samples on triplicate Baird Parker agar plates. All plates were incubated at 37°C for 24h, and then colonies were enumerated and expressed as CFU/ml.

5. Determination of D-values

D_{68}°C-values for heat shocked and non heat shocked cells were determined in Baird Parker agar plates. D-values were determined taking the negative reciprocal of slope of the best fitted line.

6. Calculation of heat shock ratio

The effect of heat shocking was evaluated by a heat shock ratio that determined as follows **(Linton et al 1992).**

$$\text{Heat shock ratio} = \frac{\text{Heat shock D-value}}{\text{Non heat shock D-value}}$$

7. Protein extraction and electrophoretic analysis

Proteins were extracted from heat and non-heat shocked cells. One-dimensional SDS-PAGE was performed as described by **(Barakat et al 2017).** The molecular weight of protein profile was estimated in comparison with standard molecular weight markers (Sigma, USA). A high-molecular-protein marker that produced bands at 135, 100, 90, 63, 48, and 35 kDa and a low-molecular-protein marker that give bands at 25, 20 and 11 kDa were used. The protein bands were visualized by staining with Coomassie Brilliant Blue R-250 (Sigma, USA) for 0.5-2 hours and were analyzed by using gelanalyzer2010 software.

8. Scanning Electron Microscopy

For Scanning Electron Microscopy, bacteria were grown in a liquid media to the exponential phase and harvested by centrifuging at 14000 rpm for 8 min. The cells were fixed at 24°C for 60 min with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.2, dehydrated with a serial concentration of ethanol, and then dried. The dried cell samples were coated with gold and examined using a scanning electron microscope JOEL- JSM 5200 **(Knutton, 1995).**

9. Statistical analysis

Data were statistically analyzed by **SAS (2009)** for significant differences by analysis of variance using Duncan´s multiple range test at significant level P≤0.05 **(Duncan,1955).**

RESULT AND DISCUSSION

1. Impact of the sublethal heat stress on development of the thermotolerance

The effect of heat shock time and temperature on survival of *S. aureus* at 68°C/ 15 min was shown in **Table 1.** Bacterial cells that were subjected to a heat shock of 60°C for 30 min had a greater resistance to a subsequent heat treatment at 68°C for 15 min followed by heat shocked cells at 47°C for 5 min, 57°C for 15 min and 52°C for 30 min. In fact, 60°C for 30 min resulted within the greatest percent of survivors to the following heat treatment than the other temperature (75.1%).

Results are in harmony with **Cebrián et al (2010)** who mentioned that exposure of exponential growth phase cells of *S. aureus* to sublethal heat from 40 to 48°C for 5-120 min resulted development of resistance to 58°C. Exposure cells to sublethal temperatures induce higher thermotolerance for various species, together with *E. coli, L. monocytogenes, Yersinia enterocolitica, lactobacillus plantarum* and *S. aureus* **(Singh et al 2007).**

Table 1. Effect of heat shock time and temperature on survival of *S. aureus* at 68°C/ 15min

Heat shock treatment [a]		
Temperature (°C)	**Time (min)**	**Viability (%)**
47	**5**	**73.9**
	15	**66.9**
	30	**66**
	60	**69.2**
52	**5**	**66.7**
	15	**67.9**
	30	**69.4**
	60	**68.5**
57	**5**	**67.4**
	15	**72.1**
	30	**66.3**
	60	**68.1**
60	**5**	**57.1**
	15	**65.4**
	30	**75.1**
	60	**67.9**

[a] Data represent the average of three separate experiments under the same conditions

2. Determination of D_{68} –values heat shocked

Heat shocking significantly increased the D_{68} – values of *S. aureus* **(Table 2 and Figure 1)**. There was a significant difference ($p<0.05$) in D-values between heat shocked and control cells. D_{68} – values of *S. aureus* were much higher when subjected to heat shock conditions, and non-heat shocked cells (4.83 min). When a heat shock was performed at 47 ° C for different times (5 - 60 min), D_{68} values recorded 5.43 to 8.83 min, the increase ranged from 1.12 to 1.83 folds.

While, exposure of microbial cells to sublethal heat stress at 52° C for different times (5 - 60 min), the higher D_{68} values to be 6.63 to 10.23 min, the increase in the D_{68} values was 1.37 to 2.12 fold. The same trend was also observed for heat shocked cells at 57 and 60°C The highest D-value

at 68°C was 10.23 min for heat shocked cells at 52°C for 30 minutes. These results are in accordance with those of **Li et al (2017).**

The ratio of heat shocked cells D-value of all treatments ranged from 1.12 to 2.12. A calculated heat shock ratio greater than 1.0 indicated that heat shocking increased the thermal tolerance **(Linton et al 1992). MCmahon et al (2000)** observed that D_{55}-values for heat-shocked *Y. enterocolitica* at 45°C for 30 min were five times greater than the non-heat-shocked cells D_{55}-values.

Table 2. D-values (min) and heat shock ratio (%) at 68°C for 15 min

Temp. / Time	D-value cont.	D-value at 47°C	Heat shock ratio (47°C)	D-value at 52°C	Heat shock ratio (52°C)	D-values at 57°C	Heat shock ratio (57°C)	D-value at 60°C	Heat shock ratio (60°C)
5min	4.83^{aE}	5.43^{dD}	1.12	6.63^{cC}	1.37	8.03^{bA}	1.66	7.43^{aB}	1.54
15min	4.83^{aC}	6.83^{bB}	1.41	7.23^{bA}	1.50	7.03^{cA}	1.46	6.73^{cB}	1.39
30min	4.83^{aD}	8.83^{aB}	1.83	10.23^{aA}	2.12	8.83^{aB}	1.83	6.93^{bC}	1.43
60min	4.83^{aD}	7.63^{bA}	1.58	7.03^{bB}	1.46	6.23^{dC}	1.29	5.83^{dC}	1.21

Means followed by different little letters within the same column (effect of time) are significantly different (P≤0.05).

Means followed by different capital letters within the same row (effect of heat temperature) are significantly different (P≤0.05).

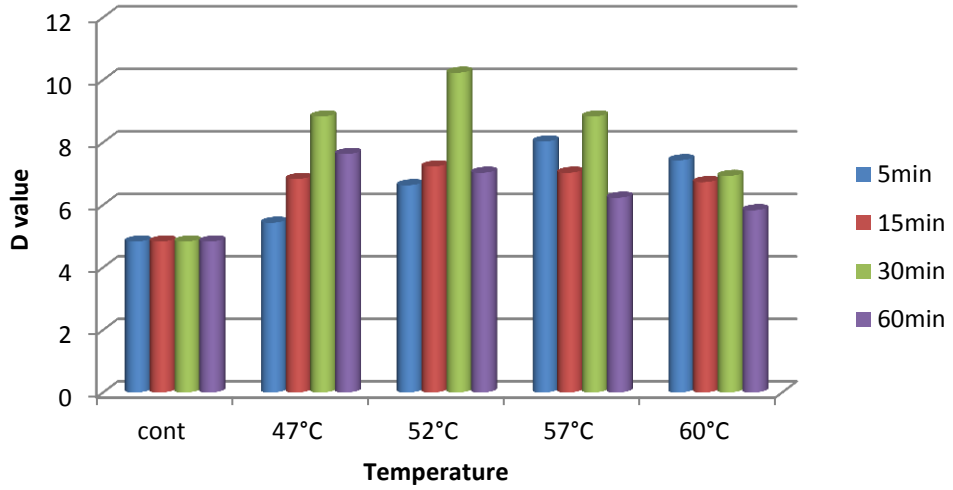

Figure 1. D-values at 68°C/15 min

Heat shocking of *S. aureus* survivors increased to a consequent heat treatment. The D- value of heat-shocked grown cells was 2.12 folds and was more than of the non heat shocked grown one. Heat shocking that had such a positive effect on the survival of microorganism cells to a consequent heat treatment is consistent with results according to **Knabel et al (1990).**

3. SDS – PAGE profile of heat shocked cells

Thermotolerance of heat-shocked cells usually has been associated with the production of heat-shock or stress proteins **(Murano and Pierson, 1992).** Approximate molecular weights and percentage of induced as well as disappeared proteins in response to different heat shock treatments were summarized with respect to detectable changes occurred in percentages of other proteins **(Tables 3-6 and Figure 2).**

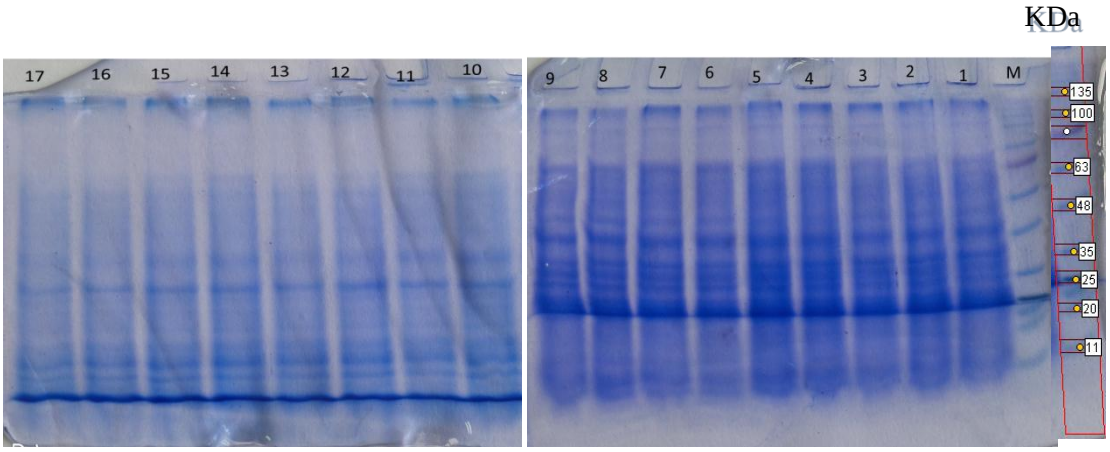

Figure 2. SDS-PAGE analysis of heat shocked *S. aureus* cells

Lanes 1 and 17: marker(M); 1: control; 2: heat shocked (47°C/5min); 3 heat shocked (47°C/15min); 4: heat shocked (47°C/30min); 5: heat shocked (47°C/60min); 6: heat shocked (52°C/5min); 7: heat shocked (52°C/15min); 8: heat shocked (52°C/30min); 9: heat shocked (52°C/60min); 10: heat shocked (57°C/5min); 11: heat shocked (57°C/15min); 12: heat shocked (57°C/30min); 13: heat shocked (57°C/60min); 15: heat shocked (60°C/5min); 15: heat shocked (60°C/15min); 16: heat shocked (60°C/30min); 17: heat shocked (60°C/60min).

Data indicated that 8 - 11 new bands representing 54.3 - 90.09 % of total cell proteins that induced to heat shock treatment at 47°C for 5-60 min. Furthermore, induced HSPs with molecular weights of 135 and 27 KDa, the predominant ones represent more than 15 % of induced proteins **(Table 3).**

On the other hand, 4 – 9 bands (representing 35.28 – 85.69 % of total cell proteins) were disappeared because of heat shock treatment. Similar trend was also observed for other investigated heat shock treatments. Among the disappeared bands, proteins having molecular weights of 140, 56 and 28 KDa were disappeared from all heat shocked samples **(Tables 3-6).** All heat shock treatments were able to induce heat shock proteins.

Furthermore, other considerable changes in protein composition of *S. aureus* due to previously treatments were recorded. Expression of 1–6 bands (visualized in control) were increased and / or decreased in response to examined heat shock treatments by 13.92 –51.18%, respectively. These results are in agreement with **(Urban-Chmiel et al 2013 and Li et al 2017).**

Schumann (2003) and (2007) showed that increasing of thermo-tolerance is expounded to the heat shock protein induction. for instance, a 25 min heat shock at 46 °C for *E. coli* O157:H7 leads to a rise of the 69 kDa DnaK and 60 kDa GroEL proteins **(Juneja et al 1998),** that are classified as HSP70 and HSP60, severally **(Tobian et al 2004).** When *E. coli* subjected to 41.5°C for 2 h heat shock, their HSP70 and HSP60 were clearly accumulated **(Urban- Chmiel et al 2013).** The role of proteins is to safeguard cells against high temperatures and to assist in recovery when stress is removed **(Richter et al 2010).**

Consequently, it's necessary to work out the enhanced heat resistance of pathogens as affected by varied pre-treatment conditions because of the induction and synthesis of HSPs. During heat shock, proteins become partially denaturated, exposing the hydrophobic regions, which then interacted to form insoluble aggregates. By binding tightly to hydrophobic surfaces, HSP 70 limits such interactions and promotes disaggregations **(Pelham, 1986).**

Table 3. Changes in protein composition of *S. aureus* in response to heat shock at 47°C.

Treatment	Molecular weights (KDa) and percentages of		
	Induced proteins	Disappeared proteins	Other associated changes
47°C for 5 min	135 (5.29), 66 (6.16), 54 (8.49), 41 (7.29), 27 (7.75), 25 (6.29), 15 (7.24) and 14 (6.51).	140 (7.54), 56 (9.60), 43 (9.52) and 28 (8.62).	88 (+0.5), 83 (-1.65), 55 (-2.50), 44 (-0.88), 22 (+1.6) and 11 (+1.71).
47°C for 15 min	135 (4.77), 103 (6.56), 66(7.20), 54 (9.65), 42 (7.78), 41 (8.87), 27 (7.95), 15 (8.68) and 10 (8.55).	140 (7.54), 83 (9.84), 56 (9.60), 44 (11.33), 43(9.52), 28 (8.62) and 11 (9.41).	88 (+1.47), 55 (-4.14) and 22 (+2.30).
47°C for 30 min	135 (6.16), 123 (4.74), 86(6.96), 65 (6.13), 60 (6.75), 57 (7.44), 52 (8.49), 42 (9.54), 27 (7.25), 25 (7.29) and 10 (8.13).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55(13.03), 44 (11.33), 43 (9.52) and 28 (8.62).	22 (+1.9) and 11 (-1.05).
47°C for 60 min	135 (5.65), 85 (8.32), 65 (6.82), 57 (7.62), 50 (11.38), 42 (8.82), 31 (11.53), 27 (8), 24 (5.97), 18 (6.60) and 10 (9.43).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55(13.03), 44 (11.33), 43 (9.52), 28 (8.62) and 11 (9.41).	22(-2.17).

Table 4. Changes in protein composition of *S. aureus* in response to heat shock at 52°C.

Treatment	Approximate molecular weighs (KDa) and percentages of		
	Induced proteins	Disappeared proteins	other associated changes
52°C for 5 min	130 (4.93), 85 (7.98), 71 (5.97), 57 (9.25), 50 (11.26), 41(8.45), 31 (8.72), 27 (7.97), 24 (6.50), 16 (6.80) and 10 (9.61).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55(13.03), 44 (11.33), 43 (9.52), 28 (8.62) and 11 (9.41).	22 (-0.06).
52°C for 15 min	130 (5.17), 104 (3.94), 85 (7.13), 65 (5.08), 56 (4.40), 53 (6.20), 50 (8.02), 41 (8.93), 31 (4.56), 27 (6.32), 24 (5.42), 19 (4.53), 17 (6.44), 15 (5.02) and 10 (5.86).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 44 (11.33), 43 (9.52), 28 (8.62) and 11 (9.41).	22 (+0.83)
52°C for 30 min	125 (7.06), 86 (6.46), 65 (5.92), 61 (8.06), 57 (6.94), 47 (7.74), 40 (8.53), 33 (8.74), 27 (8.59), 19 (7.40), 15 (6.62) and 9 (7.08).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55(13.03), 44 (11.33), 43 (9.52), 28 (8.62) and 11 (9.41).	22 (-0.81).
52°C for 60 min	125 (6.96), 86 (6.72), 71 (4.99), 63 (8.26), 57 (7.59), 47 (6.85), 40 (7.89), 33 (7.95), 27 (7.98), 23 (7.83), 19 (5.50), 15 (5.02), and 10 (7.67).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55(13.03), 44 (11.33), 43 (9.52), 28 (8.62) and 11 (9.41).	22(-2.4).

Table 5. Changes in protein composition of *S.aureus* in response to heat shock at 57°C.

Treatment	Approximate molecular weighs (KDa) percentages of		
	Induced proteins	Disappeared proteins	other associated changes
57°C for 5 min	102 (3.30), 98 (4.47), 79(6.40), 67 (5.54), 57 (6.03), 52 (4.83), 50 (5.92), 46 (5.45), 40 (5.72), 36 (6.95), 31 (5.54), 29 (5.12), 27 (4.82), 26 (4.70), 24 (7.13) and 23 (7.44).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 44 (11.33), 28 (8.62), 22 (14.26) and 11 (9.41).	43 (+1.96).
57°C for 15 min	102 (7.24), 82 (7.47), 72(6.81), 57 (8.86), 52 (9.42), 40 (6.93), 36 (7.69), 32 (6.19), 31 (6.39), 30 (5.79), 27 (9.61) and 24 (7.57).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 44 (11.33), 28 (8.62), 22 (14.26) and 11 (9.41).	43 (-1.86).
57°C for 30 min	102 (5.65), 86 (6.95), 73(6.56), 60 (9.09), 52 (7.21), 41 (7.37), 36 (7.42), 32 (9.03), 30 (7.99), 27 (6.32) and 24 (9.65).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 44 (11.33), 28 (8.62) and 11 (9.41).	43 (+0.39) and 22 (-8.78).
57°C for 60 min	102 (5.16), 86 (6.51), 71(6.53), 59 (5.58), 53 (7.42), 51 (9.09), 41 (6.82), 36 (6.15), 32 (8.94), 30 (8.38), 27 (10.60) and 24 (9.39).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 44 (11.33), 28 (8.62), 22 (14.26) and 11 (9.41).	43 (-1.15).

Table 6. Changes in protein composition of *S.aureus* in response to heat shock at 60°C.

treatment	Approximate molecular weighs (KDa) and percentages of		
	Induced proteins	Disappeared proteins	Other associated changes
60°C for 5 min	102 (5.89), 86 (6.26), 70 (5.88), 64 (5.27), 53 (5.35), 51 (5.80), 49 (6.89), 40 (5.66), 36 (8.39), 32 (7.09), 31 (7.58), 27 (11.32) and 24 (9.54).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 44 (11.33), 28 (8.62), 22 (14.26) and 11 (9.41).	43(-0.65).
60°C for 15 min	102 (10.55), 85(5.40), 72(6.40), 60 (6.36), 52 (7.17), 50 (6.91), 41 (7.01), 36 (7.70), 32 (6.47), 31 (6), 29 (6.69), 26 (9.15) and 24 (8.95).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 44 (11.33), 28 (8.62), 22 (14.26) and 11 (9.41).	43 (-1.01).
60°C for 30 min	89 (6.51), 71 (7.89), 60 (6.31), 54 (6.99), 50 (9.10), 36 (8.11), 32 (8.22), 31 (4.15), 29 (7.28), 26 (10.23) and 24 (8.68).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 43 (9.52), 28 (8.62) and 11 (9.41).	44 (-3.68) and 22 (-8.82).
60°C for 60 min	85 (8.08), 73 (8.26), 65 (8.39), 58 (7.34), 53 (6.13), 51 (7.62), 48 (7.53), 40 (7.14), 33 (8.41), 29 (7.92), 25 (9.98), 24 (9.03) and 21 (4.11).	140 (7.54), 88 (6.80), 83 (9.84), 56 (9.60), 55 (13.03), 43 (9.52), 28 (8.62), 22 (14.26) and 11 (9.41).	44 (-3.96).

4. Scanning Electron Microscopy of bacterial cell wall.

Heat shocked cells at 52°C/15 min and 57°C /5 min were selected as the best heat shock treatments for the induction of heat shock proteins as well as for the examination of scanning electron microscope. Examination by scanning electron microscope exhibited normal morphological shape for the control cells of *S. aureus.* While, heat shocked cells exhibited severe destruction (i.e., rupture, irregular and leakage of cell contents) **Figure (3).** However, heat shocked cells after incubation at 37°C for overnight in very enrichment medium regained to normal ones.

One of the foremost characteristics of injured microorganism cells was the flexibility for repairing injury during a suitable environment to be the same as normal cells. The injured cells will repair during a medium devoid of selective compounds throughout incubation at optimum ph and tempera-

ture. In general, cells repair well during a medium wealthy in metabolizable carbon and nitrogen sources and several other vitamins. Supplementation with enzyme and pyruvate (to destroy H_2O_2 created by the cells) additionally enhances repairing will increase the quantity of repaired cells.

Throughout fast repair process, several cells generate H_2O_2 however fail to hydrolyze it attributable to an injured peroxidase system. Accumulated H_2O_2 consequently causes death. Looking on sublethal stress, complete repair are often achieved in 1 to 6 h at 25 to 37°C. Just in case of freezing and drying injuries, the rate is extremely rapid; for heat injuries, the rate may be slow **(Ray, 1992).**

The major inducible HSP70 plays a very important role to reduce the inactivation level needed for microbiological food safety. Determinant the heat-shock response and thermotolerance is much necessary to manage *S. aureus* in postharvest agriculture product that are heated below completely different harvesting, process and storage conditions. Therefore, the improved heat resistance of *S. aureus* because of heat shock should be thought-about, whereas designing effective thermal processes.

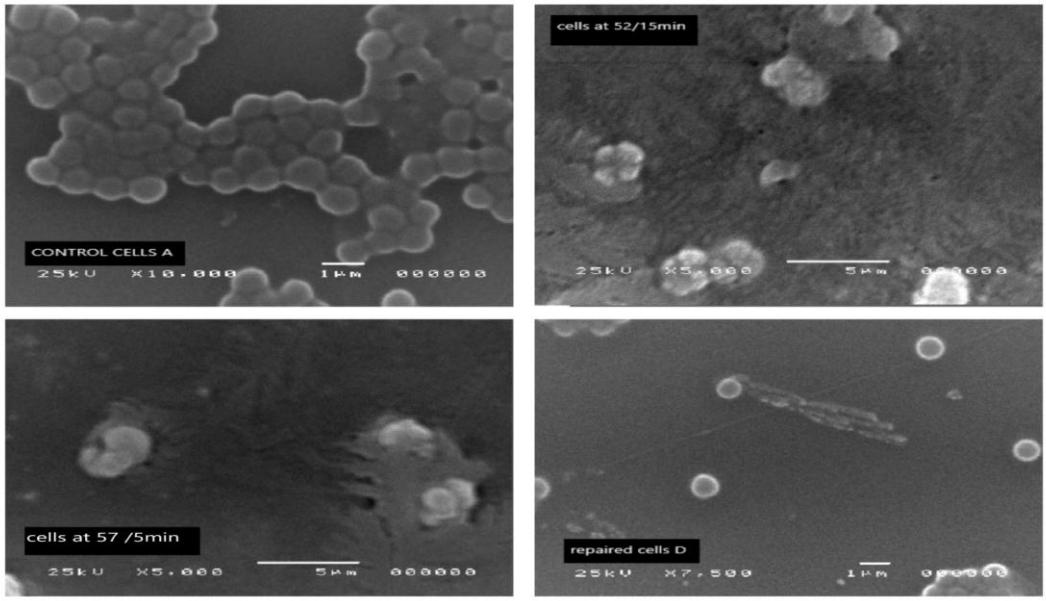

Figure 3. Cells with electron microscopy

CONCLUSION

In this study, the heat resistance of *S. aureus* in buffered peptone water at 68° C was obtained. The preheating conditions at four sub-lethal temperatures resulted a rise in heat resistance of *S. aureus* with most D-values when heat shock at 52 °C for thirty min. Maximum induced heat shock proteins with molecular weight from 23 to 102 KDa was recorded after heat shock at 57°C /5 min. Incubation of heat shocked cells at 37°C overnight in very enrichment medium showed a repair of cell wall and regained to its normal shape. Avoiding heat shock proteins or delicate temperatures or treatment times should be considered to confirm microbiological food safety; additional experiments ought to be conducted to check the thermotolerance of *S. aureus* in real foods to validate the results obtained during this study.

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المقاومة الحرارية لخلايا بكتريا المكورات العنقوديه الذهبيه عقب التعرض للصدمة الحرارية دون المميته

[43]

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الموجـــــز

تم دراسة تأثير الصدمة الحرارية المسبقة علي المقاومة الحرارية للمكورات العنقودية الذهبية في المزرعة السائلة وتخليق بروتينات الصدمة الحرارية، كما تم فحص الجدار الخلوي لخلايا المكورات العنقودية الذهبية بإستخدام تقنية الميكروسكوب الإلكتروني الماسح. وقد تم تنمية البكتريا علي درجة الحرارة المثلى 37°م (العينة الضابطة). وفي هذه الدراسة تم إجراء صدمة حرارية دون المميتة لخلايا البكتريا علي 47، 52، 57 و60°م لأزمنة مختلفة ثم تم إجراء معاملة حرارية علي 68°م. وأوضحت النتائج أن متوسط قيمة D_{68} للعينة الضابطة سجلت 4.83 ق في حين تراوحت قيم D_{68} للعينات المعرضة للصدمة الحرارية دون المميتة من 5.43 إلي 10.23 ق. كما أظهرت نتائج تقنية الفصل في المجال الكهربائي تكوَّن 8-16 بروتين نتيجة التعرض لصدمات الحرارية دون المميتة لخلايا المكورات العنقودية الذهبية وكانت أوزانها الجزيئية تتراوح من 9 إلي 135 كيلو دالتون. كما وجد أيضاً أن زيادة المقاومة الحرارية للخلايا الميكروبية كان متزامناً مع ظهور هذه البروتينات المخلقة. وقد أظهر فحص الخلايا الميكروبية بإستخدام تقنية الميكروسكوب الإلكتروني الماسح أن معاملات الصدمة الحرارية دون المميتة أدت إلي أضرار شديدة علي الجدار الخلوي (مثل تحطم الجدار الخلوي، ظهور الخلايا بشكل غير منتظم وخروج المحتويات الخلوية) في حين أن تحضين الخلايا الميكروبية في البيئة الغنية بالمغذيات علي درجة 37°م /12 ساعة ساهم بدرجة كبيرة في إصلاح الأضرار التي لحقت بالجدار الخلوي وأصبحت مماثلة للعينة الضابطة. وخلُصت الدراسة إلي أنه يجب الأخذ في الإعتبار زيادة المقاومة الحرارية للمكورات العنقودية الذهبية نتيجة التعرض للصدمة الحرارية دون المميتة عند تصميم المعاملات الحرارية الفعالة لضمان السلامة الميكروبيولوجية للغذاء

كلمات دالة: المقاومة الحرارية، بكتريا المكورات العنقودية، بروتينات الصدمة الحرارية، الفحص الميكروسكوبي

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