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Thermal Death Time of Staphylococcus Aureus (PTCC=29213) and Staphylococcus Epidermidis (PTCC=1435) in Distilled Water

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Abstract: Due to the frequent presence of Staphylococcus aureus and Staphylococcus epidermidis in human, animal and food stuffs, in this study, Thermal Death Time of these two species was studied and then compared together. 1 ml of the microbial suspension was added to each of sterile 3 ml extent plastic tubes and then, these tubes was transmitted in to the 55, 58, 62, and 65 ^{o}C water bath and all of them was tested at times 0, 5, 10, 15, 20, 25, and 30 minute. Lastly, it was cultured and controlled with colony forming or without it, after 48 hours incubation in 37 ^{o}C. According to the results, TDT of S. aureus was 32.5, 22.5, 12.5, 7.5 minute and for S. epidermidis was 27.5, 17.5, 7.5 and 2.5 minute, respectively in these temperatures. Comparisons of mean TDT of two species in the related temperatures showed significant difference between TDT of two species in low temperatures (55 ^{o}C, p=0.001 and 58 ^{o}C, p=0.045), but there wasn't any significant difference in high temperatures (62 ^{o}C, p=0.401 and 65 ^{o}C, p=0.18). So, it was concluded that thermal resistance of S. aureus significantly is higher than S. epidermidis in low temperatures without any significant difference in high temperatures.

Key words*:* Staphylococcus aureus, Staphylococcus epidermidis, Thermal Death Time.

INTRODUCTION

Genus Staphylococcus is the cause of many different disease in human and animals, furthermore it is one of the most important bacteria in food hygiene and the serious problem caused by Staphylococcus species is food intoxication to be created with enterotoxins of this organisms (Bergdoll, 1989; Varnam and Evans, 1996; Banwart, 1998; Jay *et al*., 2005).

One of the usual methods for elimination of these organisms is thermal treatment and if applied properly, could be effective way for improving microbial properties of food. So, study on Thermal Death Time (TDT) of this bacteria to achieve a proper thermal processing is necessary (Webster and Esselen, 1956; Angelotti, *et al*., 1961; Busta and Jezeski, 1963; Thomas *et al*., 1966; Jay *et al*., 2005). Thermal Death Time is the time needed to kill a given number of organisms at a specified temperature (Buffo and Holley, 2006). This study included three main purposes as follows:

1) Determination of TDT of Staphylococcus aureus as a representative of coagulase positive Staphylococcus on specified temperatures under the pasteurization heat (55^{o}C to 65^{o}C), in distilled water.
2) Determination of TDT of Staphylococcus epidermidis as a representative of coagulase negative Staphylococcus at heat range and condition mentioned above.
3) Comparison of mean TDT of S.aureus and S.epidermidis at same temperatures and conditions and comparison of thermal resistance of them with together.

MATERIALS AND METHODS

Firstly microbial suspension provided for both of the Staphylococcus aureus (PTCC=29213) and Staphylococcus epidermidis (PTCC=1435) separately, which it's concentration was 1.5×10^{8} CFU/ml of distilled water base on 0.5 MacFarland tube turbidness (Figure: 1), (McFarland, 1907). It was added 1 ml of the microbial suspension to each of sterile 3 ml content plastic tubes and then, these tubes was transmitted in to the 55, 58, 62, and 65^{o}C water bath and all of them was tested at times 0, 5, 10, 15, 20, 25, and 30 minute. Lastly, it was cultured in the Baird parker agar medium (Merck), as surface cultured method and then it was controlled with colony forming or without it, after 48 h incubation in 37 ^{o}C. (All of stages were repeated 6 times for each of two species). For data analyzing T-test and Mann-Whitney test were used. Growth-No-Growth method was used to draw TDT curves.

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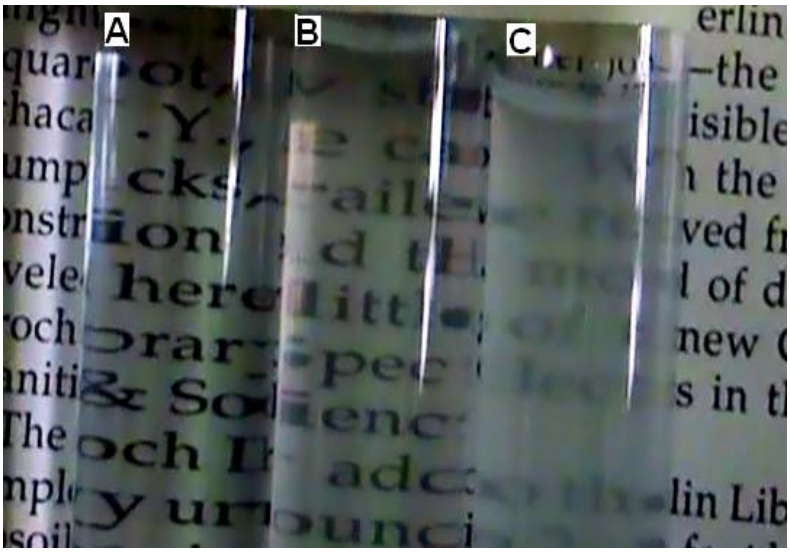

Fig. 1: MacFarland tube turbidness, A: Distilled Water, B: 0.5 MacFarland tube turbidness, C: 1.0 MacFarland tube turbidness

RESULTS AND DISCUSSIONS

TDTs formula of S.aureus and S.epidermidis in four tested temperatures are presented in Table (1), TDT curves obtained for these two species showed in Figure (2), and mean TDTs calculated for statistical comparison of these species in four tested temperatures are summarized in Table (2).

Table 1: TDTs formula of S.aureu and S.epidermidis on the basis of results.

S.epidermidis	S.aureus
TDT_{55} = 27.5'	TDT_{55} = 32.5'
TDT_{58} = 17.5'	TDT_{58} = 22.5'
TDT_{62} = 7.5'	TDT_{62} = 12.5'
TDT_{65} = 2.5'	TDT_{65} = 7.5'

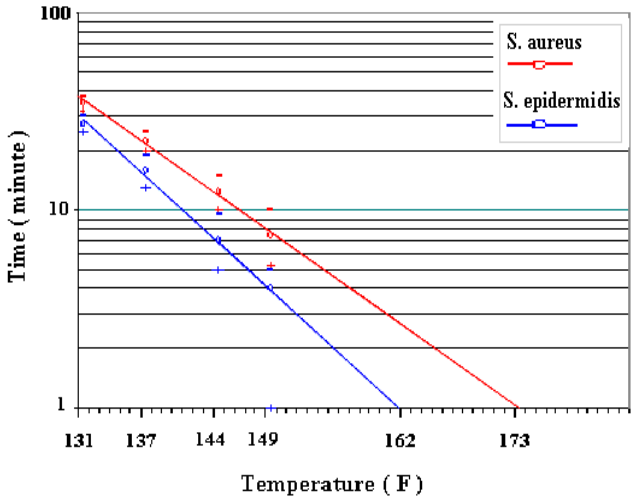

Fig. 2: TDT curves of S.aureu and S.epidermidis as logarithmic with Growth-No-Growth method. (temperatures are a arithmetical numbers and times as minute).

Table 2: Mean TDT of S.aureus and S.epidermidis.

Mean TDT	S.aureus	32.33	19.83	8.17	6.50
	S.epidermidis	23.17	13.17	6.50	4.00

Discussion:

Results indicate that there is highly significant difference between mean TDT of organisms in different temperatures ($p<0.01$), except 62 and 65°C and there is not significant difference between these two temperatures (p=0/474 for S.aureus, p=0/163 for S.epidermidis), it mean that cell injury mechanisms as damage to DNA, RNA or cytoplasmic membrane to be completed in 62°C and it is sufficient for killing bacteria (Dilella and Sobota, 1980).

TDT curves of two species that obtained as straight line shows that death pattern of each bacteria is logarithmic. Slope of TDT curve of S.aureus is less than S. Epidermidis (Figure. 2), so it is concluded that thermal resistance of S.aureus is more than S.epidermidis (Frazier, 1958). Regard to TDT curves, maximum times that S. Epidermidis can endure in 4 tested temperatures is equal with minimum times that S. Aureus can endure.

TDT of S.aurus in 60° C was explained 18.8 minute that is approximately same as our results (Frazier, 1958). It was reported that Staphylococcus aureus Strain C12069 (Dolman) survive heating for more than 30 minutes at 55° C (8.2 per cent) in cream filling (pH: 6.48), also 8 minutes of heating at 65° C, less than 4 minutes of heating at 75 ° C, and less than 3 minutes of heating at 85° C, kills this strain of organism in cream filling (Hussmann and Tanner, 1947).

TDTs of S.aureus MS149 strain at 54.4, 57.2, 60, 62.7, 65.5°C in Custard were explained respectively 530, 165, 53, 16.5, 5.2 minute , and in chicken a laking 540, 130, 40, 11.5, 3.4 minute with primary number of 1×10^7 CFU/gr.

Also TDTs of S. Aureus 196E strain at these temperatures were expressed respectively 540, 180, 59, 19.5, 6.6 minute in Custard, and 425, 140, 47, 15.5, 5.2 minute in chicken with the same number of organism. TDTs of MS149 strain in Ham salad at 54.4, 60, 65.5°C were explained respectively 200.0, 40.0, 6.0 minute with the same number of organism (Angelotti *et al*., 1961). Results of this research are nearly same to our results just at high temperatures (Table: 1).

It was showed that viable cells of several strains of S.aureus in frankfurters heated to 160 degrees F (71.1°C) were undetectable (Palumbo *et al*., 1977).

It was indicated that S.aureus is more thermotolerant than Listeria monocytogenes and should be used as the target microorganism in designing mild thermal treatments for food, and they recommended 70°C for 2 minute (minimum) for pasteurization (Kennedy *et al*., 2005).

Comparisons of mean TDT of two species in the related temperatures (Table: 2) showed significant difference between TDT of two species in low temperatures (55° C, p=0.001 and 58° C, p=0.045), but there wasn't any significant difference in high temperatures (62° C, p=0.401 and 65° C, p=0.18).

Conclusion:

It was concluded that thermal resistance of S.aureus significantly is higher than S.epidermidis in low temperatures without any significant difference in high temperatures. Furthermore, present research indicate that 62^o C for 12.5 minute in Staphylococcus aureus and 7.5 minute in Staphylococcus epidermidis is sufficient to complete destruction of microorganism in distilled water.

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