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**Original article**

# Variations in growth of *Staphylococcus aureus* 234 after heat stress in milk

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**Summary** – The cells of *S aureus* 234 after heat stress exhibited extended lag phase. The length of lag phase was directly related to the intensity of the heat treatment. Mild heat treatment (50 °C) of *S aureus* 234 did not result in appreciable change in the lag phase. However, the lag phase was extended to 2 and 4 h, respectively, in cells stressed at 55 and 62.5 °C. The addition of salt (5.0–7.5%) and tellurite (0.2–0.4%) to the recovery medium further lengthened the lag phase and also reduced the growth rate.

***Staphylococcus aureus* / heat-treatment / growth / lag phase / milk**

**Résumé — Variations de la croissance de *Staphylococcus aureus* 234 dans le lait après un choc thermique.** *Les cellules de* S aureus *234 montrent, après un choc thermique, un allongement de leur phase de latence. La longueur de la phase de latence est directement reliée à l'intensité du traitement thermique. Un traitement thermique doux (50 °C) de* S aureus *234 ne conduit pas un changement appréciable du temps de latence. En revanche, ce temps s'allonge à 2 et 4 h, respectivement pour des cellules ayant subi un choc thermique de 55 et 62,5 °C. L'addition de sel (5,0–7,5%) et de tellurite (0,2–0,4%) au milieu de recouvrement allonge encore plus le temps de latence et réduit aussi le taux de croissance.*

**Staphylococcus aureus / *traitement thermique / croissance / phase de latence / lait***

## INTRODUCTION

The role of *S aureus* in food poisoning outbreaks through milk and milk products is well documented by virtue of the ability of some strains to produce highly heat resistant enterotoxins in the incriminated foods (Ghose and Chattoraj, 1963; Bergdoll, 1979; Palumbo and Smith, 1984). During processing, *S aureus* present in foods is subject to various stress conditions such as heating, freezing and vacuum concentration, etc. However, some of the milk products like dried and concentrated milks receive only a mild form of heat treatment which may be insufficient to kill off all *S aureus* cells. Such sublethal heat

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lethal heat treatment may leave residual staphylococci which have survived heating for a long period of time (Batish *et al,* 1989, 1990). Hence, it needs to be ascertained whether such staphylococci, subjected to sub-lethal heat treatment, differ in any way from their unheated counterparts. In this connection, the variation in the growth pattern of such stressed cells of *S aureus* in processed foods assumes greater significance, as the same is likely to adversely affect the important metabolic activities of this organism, used for identification purposes in quality control laboratories. This study was undertaken to determine the growth pattern of enterotoxigenic *S aureus* 234 after heat stress. The effect of heat stress on enterotoxin production, thermonuclease activity and other biochemical characteristics of this strain have been determined and reported previously (Batish *et al,* 1989).

## MATERIALS AND METHODS

*S aureus* 234 used in the present study was obtained from GK Murthy of the Food and Drug Administration, USA, and was maintained on nutrient agar slants with fortnightly subculturing. The culture was subjected to heat treatment in cow and buffalo milk and surviving staphylococci were recovered on soya bean casein digest (SCD) agar medium as reported previously (Batish *et al,* 1989). For further studies, one of the colonies from the staphylococci surviving each heat treatment was taken and transferred to BHI broth along with the parent (unheated) culture.

Growth behaviour of *S aureus* 234 before and after heat stress in milk (50, 55 and 62.5 °C for 30 min) was determined separately by inoculation at the rate of approximately 500–1 000 cells/ml in BHI broth (100 ml) using 250-ml flasks supplemented separately with 0, 2.5, 5.0 and 7.5% sodium chloride as well as 0, 0.2 and 0.4% potassium tellurite. One-ml sample aliquots were removed at different intervals during incubation at 37 °C and plated onto SCD medium. Growth curves in respect to normal and stressed *S aureus* 234 were plotted by taking time intervals in h along an X-axis and log number of colony forming units/ml on a Y-axis.

## RESULTS AND DISCUSSION

The effect of heat treatment at different temperatures for 30 min on the growth of surviving cells of *S aureus* 234 at 37 °C in BHI broth has been recorded in figure 1. The length of lag period encountered in unheated cells was approximately 1 to 1.5 h. However, this lag period was extended when the cells were stressed at different temperatures. Surviving staphylococci from higher temperature treatment exhibited a prolonged lag period (2 h at 50–55 °C *vs* 4 h at 62.5 °C). When *S aureus* 234 cells were stressed at 50 °C for 30 min, the lag period observed was 2 h, which was twice that of unheated *S aureus* 234. An almost similar trend (2 h lag phase) was observed when a colony surviving 55 °C heat treatment for 30 min was studied. Contrary to this, the lag period was extended to 4 h in the case of surviving staphylo-

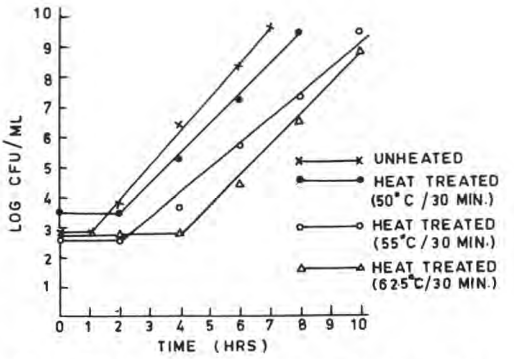


**Fig 1.** Effect of heat treatment on the growth of *S aureus* 234 in BHI broth at 37 °C.
*Effets des traitements thermiques sur la croissance de* S aureus *234 dans le milieu BHI à 37 °C.*

cocci at 62.5 °C for 30 min treatment, indicating a more deleterious effect of the heat treatment on the growth rate of the affected cells. However, after the delayed lag phase the heat stressed cells at 50 and 62.5 °C appeared to multiply more or less at the same rate as their unheated counterparts, as is evident from figure 1. The slight variation in the steepness of the slope in growth curve shown in the same figure with the staphylococci surviving the 55 °C treatment is difficult to explain. However, the possibility of the colony in question receiving a more lethal heat stress at 55 °C out of the whole population due to separation from the clump cannot be ruled out. This discrepancy may also be attributed to experimental error as the result of a single colony analysis. Nevertheless, the reduction in the extent of growth of the heat stressed colonies is clearly reflected by their extended lag phase. These findings are similar to the earlier observations of Jackson and Woodbine (1963) and Allwood and Russel (1969), who observed that heated cells when placed in recovery media and incubated at 37 °C carried out RNA synthesis at an early stage during incubation, although the rate of synthesis was considerably less than that of the unheated cells. Sogin and Ordal (1967) showed that RNA synthesis is involved in the recovery process of heat stressed *S aureus.* The prolonged lag phase in heat stressed cells of *S aureus* 234 observed during the present studies may also account for reduced or altered physiological and biochemical activities of these cells, as was observed during our previous study (Batish *et al,* 1989). The reduced extent of growth chiefly due to extended lag phase of the heat stressed cells of *S aureus* 234 observed during this investigation could explain the production of reduced levels of enterotoxin and thermonuclease by the surviving staphylococci of different strains of *S aureus* on heat treatment, recorded in our earlier study (Batish *et al,* 1989). Although heat stressed cells of *S aureus* 234 at 50° and 55 °C exhibited normal coagulase activity, there was a definite decrease in enzyme activity in the cells heat stressed at 62.5 °C. In other strains of *S aureus* such as 1151 M, there was a considerable loss of coagulase activity. These variations in the behaviour of *S aureus* under stress conditions may assume greater significance during isolation and identification of *S aureus* after recovery from processed foods. Since the colonies showing variations in their characteristics examined in this investigation were picked from a non-selective medium and if the same can be extrapolated to colonies from selective media like BPA, then it would have serious consequences, for instance a colony of very small size or non-coagulating type might give misleading results during their interpretation. This could be even more serious with BPA supplemented with plasma for coagulase activity (Hauschild *et al,* 1979) or in direct coagulase-TNase tandem test (Lachica, 1980) commonly used during the isolation and identification of enterotoxigenic *S aureus* strains from different foods. Hence, great caution should be exercised while interpreting the results based on these activities.

The data pertaining to the effect of incorporation of salt in the recovery medium on the growth of heat stressed (55 °C for 30 min) *S aureus* 234 at 37 °C have been presented in figure 2. The presence of salt in recovery medium resulted in an unusually longer lag phase of 6 h. The extension of lag phase in the case of heat stressed cells grown in BHI in the presence of 5.0 and 7.5% sodium chloride is further evidenced by an initial decrease in cell population. This was followed by almost the same growth pattern as that of the unheated organisms. Thus, there was a period of adjustment after inoculation into BHI broth during which the viable numbers de-

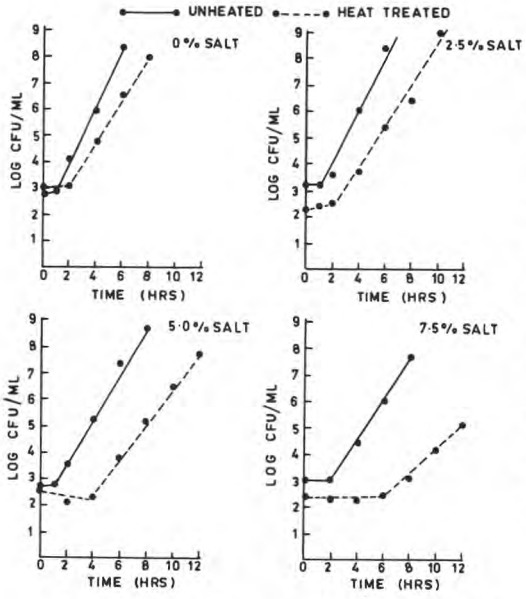


**Fig 2.** Effect of salt on the growth of stressed and unstressed cells of *S aureus* 234 in BHI broth at 37 °C.
*Effet du sel sur la croissance de* S aureus *234 dans le milieu BHI à 37 °C.*

creased slightly followed by the usual lag phase. However, the rate of multiplication of heat-injured cells in BHI broth with 7.5% sodium chloride was slower as compared to the normal cells, thereby indicating decreased salt tolerance of heat injured cells. This could be of considerable significance, since salt tolerance (7.5% sodium chloride) is considered one of the criteria for the identification of *S aureus.* Our findings in this regard are consistent with the observations of Bluhm and Ordal (1969) who also recorded that the initial inhibition of growth of stressed cells by salt present in the recovery medium was followed by the recovery of salt tolerance during the extended lag phase. Sodium chloride when ionized could exert lethal activity through osmotic effects, sodium ion toxicity or chloride ion toxicity. The salt sensitivity of heat injured *S aureus* has also been attributed to hydrogen peroxide sensitivity and impairment of catalase production during repair of the injured cells (Bucker *et al,* 1979; Bucker and Martin, 1981).

The presence of tellurite in the growth medium also resulted in an extension of the lag phase in the case of stressed cells of *S aureus* 234, as has been indicated in figure 3. The lag phase was 4 h as compared to 2 h in normal cells. In this case also, there was a decrease in the viable numbers initially followed by growth phase of the same pattern as that of unheated organisms. Hence, here also there was a period of adjustment. In this regard, we confirm the results of Hurst *et al* (1976) who

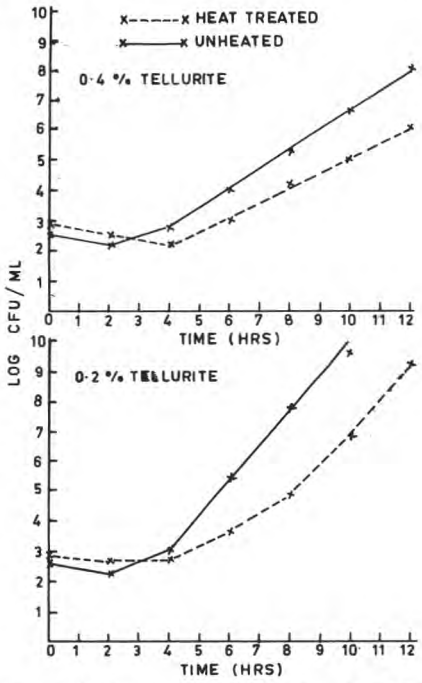


**Fig 3.** Effect of tellurite on the growth of *S aureus* 234 in BHI broth at 37 °C.
*Effet du tellurite sur la croissance de* S aureus *234 dans le milieu BHI à 37 °C.*

demonstrated the inhibitory effect of potassium tellurite on heat stressed cells of *S aureus*. As is evident from figure 3, the slope between 4 and 8 h of growth appears to be different to that of the unheated cells, although beyond 8 h the latter cells appear to follow almost the same pattern.

Hence, from the foregoing study it can be concluded that growth of *S aureus* could be definitely affected by sub-lethal heat treatments simulating milk processing conditions, particularly during the initial few hours after their recovery from heat processed foods. Hence, it is essential that, in order to avoid misleading results, such heat-stressed *S aureus* isolates should resume their normal growth through repeated subculturing before subjecting them to identification tests like coagulase, TNase and enterotoxin production.

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