Growth of *Staphylococcus aureus* and Enterotoxin Production in Blanched Mushrooms Held in Salt Brines and the Possible Role of Chitin

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Abstract: Several food poisoning outbreaks in the U.S. linked to the consumption of imported canned mushrooms have prompted studies to examine potential causes of this problem. It was shown that the presence of staphylococcal enterotoxin (SE) in the mushrooms was responsible for the outbreaks and was most likely present in the mushrooms before thermal processing. Several potential scenarios that could permit growth of *S. aureus* to sufficient populations to produce SE prior to canning have been examined. Blanched mushrooms were inoculated with enterotoxigenic type A *S. aureus* 743, packed in 7%, 14%, and 21% salt brines, stored at 30°C for 56 days, and subsequently analyzed for growth of natural microflora and S. aureus as well as the formation of SE. Enterotoxin was detected by the miniVIDAS®(fluorescent immunoassay system and SE concentration was estimated by employing a standard curve generated using purified enterotoxin. Blanched mushrooms held in 7% and 14% NaCl brines both permitted growth and enterotoxin production of *S. aureus*; the 14% NaCl considerably delayed SE production when compared to the 7% NaCl (7 days vs. 2 days, respectively). No growth or enterotoxin production was observed in the mushrooms held for up to 56 days in 21% NaCl. Addition of 14% NaCl to BHI broth also significantly inhibited growth and SE production of *S. aureus*. Blanched mushrooms held in 14% NaCl were demonstrated to be a superior growth medium for *S. aureus* than was BHI broth with 14% NaCl. These results demonstrate that some components in mushrooms apparently enhance growth and SE production of *S. aureus* under stressful conditions. The last objective of the present study was to determine the effect of chitin and chitosan on *S. aureus* growth and SE production. Although chitin added to BHI broth enhanced SE production only during the logarithmic phase of growth, the effect of chitin was more dramatic when BHI broth contained 14% NaCl. SE production occurred at < 5 days with 0.5% added chitin whereas toxin was not produced within 8 days without added chitin. These results support evidence indicating that the presence of chitin in mushrooms may predispose mushrooms to growth of *S. aureus* and to staphylococcal food poisoning outbreaks.

Key words: Canned mushrooms, staphylococcal enterotoxin (SE), salt brines, mushroom microflora, contamination, fresh mushrooms

1 Introduction

Canned mushrooms imported from the People's Republic of China (PRC) were implicated in several food poisoning outbreaks in 1989. The outbreaks occurred primarily in food-service establishments that were using the mushrooms from #10 (68oz) cans as toppings or condiments for main items.[1] The cause of the outbreaks was determined to be the presence of staphylococcal enterotoxin (SE) in the product. This led to regulatory action by the FDA to place canned mushrooms from the PRC to be placed on automatic detention and scientific investigation to determine the probable cause of these incidences. The outbreaks were considered significant because they were the first major outbreaks of staphylococcal food poisoning in the United States directly related to a canned food product[2, 3] since 1975 when canned lobster bisque soup was implicated.[4]
Studies in our laboratory demonstrated that time temperature abuse of fresh mushrooms inoculated with *S.*

aureus[5] could result in mushrooms with preformed enterotoxin (SEA). Additionally, it was determined that this enterotoxin was sufficiently heat stable to survive recommended thermal processes for canned mushrooms. Hence, these results indicated that a possible scenario for the PRC situation could be contamination of fresh mushrooms with *S. aureus* from harvesters followed by time-temperature abuse of mushrooms between the farms and the canning factories. This seemed to be a plausible explanation since large distances are common between the farm and factories and the lack of refrigeration in the transport system.

Another possible problem in the PRC system is the use of salt brines to hold blanched mushrooms prior to canning. The system involves blanching mushrooms and then packing them in heavy salt brine (near saturation > 25%) in large plastic barrels. These products are held at ambient temperature and can be held for many months and shipped large distances prior to desalting and then canned. Another practice used during periods of peak production is to hold mushrooms at the canning factories in lighter salt brines (5-15% salt) at ambient temperature for several days prior to canning. This practice occurred whenever the supply of fresh mushrooms arriving at the plant exceeded processing capacity and was used in lieu of refrigerated storage.

Both of these practices that involve holding blanched mushrooms in salt at ambient temperature could be problematic. Since *S. aureus* is salt-tolerant, it could grow if present with less competition and produce toxin that would likely survive canning. Hence, this study was initiated to investigate the potential for growth of *S. aureus* and enterotoxin production in blanched mushrooms held in salt brines with various concentration of salt. Also, past research in our laboratory indicated that the presence of chitin in mushrooms may be linked to enhanced enterotoxin production by *S. aureus* using *in vitro* studies in BHI broth.[6] Hence, another objective of this study was to investigate if chitin influences growth of *S. aureus* and SE production in BHI Broth with 14% salt present.

2 Materials and Methods

2.1 *Staphylococcus aureus* culture

Staphylococcus aureus 743 was obtained from Reginald Bennett of the Division of Microbiological Studies of the FDA (Washington, D.C.). The strain is known to produce staphylococcal enterotoxin A (SEA), which is the enterotoxin serotype most commonly associated with food poisoning, and was isolated from a food poisoning outbreak involving scrambled eggs. Cultures were maintained on trypticase soy agar (TSA; Difco Laboratories, Detroit, MI) slants and were transferred to fresh slants every two months. The slants were inoculated, allowed to incubate at 35°C for 24 hours, and then stored at 4°C until needed.

2.1.1 Inoculum preparation

A loopful of *S. aureus* 743 was aseptically transferred to 10 ml of sterile brain heart infusion broth (BHI; Difco) and incubated at 37°C for 18-20 hr. 0.5 ml of this broth was aseptically transferred into 100 ml of sterile BHI and placed in an agitating water bath (150 rpm) at 37°C for 18-20 hr. Following the shaking step, the solution was aseptically transferred to a sterile, plastic centrifuge bottle and centrifuged at 10,000 g for 20 min. at 4°C (IEC B20-A Centrifuge). The resulting supernatant was decanted and the cell pellet was resuspended with 100 ml of sterile, cold 0.1 M potassium phosphate buffer (pH 7.0) and centrifuged again. The washing step was performed twice to ensure than only *S. aureus* cells, and no staphylococcal enterotoxin, was present in the initial inoculum.

2.2 Mushrooms

Mushrooms used in this study were a hybrid off-white (U-1) strain of *Agaricus bisporus* and were obtained

from a University-operated mushroom growing facility. The mushrooms were stored for approx. 24 hr in 4°C immediately after harvesting, prior to inoculation and storage.

2.3 Growth and enterotoxin production of *S. aureus* in blanched mushrooms held in NaCl brine at 30°C

2.3.1 Preparation and inoculation of salt-brined mushrooms

Mushroom stipes were trimmed to within 5mm of the cap and an appropriate amount was then blanched by submersion in boiling water for 5 min. Immediately following blanching, the mushrooms were transferred to one of two cooling water buckets held for 24hr at 4°C. The cooling water contained either sterile water (control) or water inoculated with *S. aureus* 743 at a final cell concentration of approximately 10^4 CFU/g mushroom. Following the cooling period, the mushrooms were drained for 5 min. An appropriate amount of mushrooms was transferred into stainless steel buckets containing sterile NaCl brine at either 7%, 14%, or 21% (w/v) concentrations. The containers were then placed in a 15°C storage area to allow for equilibration. The salt concentration was measured by using a hydrometer (salometer) calibrated at 15°C to obtain a reading from a sample of each of the uninoculated brine solutions. This step was performed as aseptically as possible to reduce the risk of contamination of the control brines. Salometer readings, NaCl concentrations derived from $AgNO_3$ titration, and a_w values of 7%, 14%, 21% salt-brines after equilibration with blanched mushrooms are shown in Table1A. Since mushrooms are able to absorb solutes from their environment, the NaCl level in each of the buckets was monitored every 2 hr and adjusted as needed with the addition of appropriate amounts of NaCl until equilibrated NaCl concentrations of 7, 14, and 21% were achieved. Equilibration was considered complete when no decline in NaCl concentration in the brine was evident over a 48-hour period.

Once each container had reached equilibration, the contents were divided into clean, sanitized 1 lb. capacity plastic cream-cheese containers. Into each plastic container 200 ml of brine and approximately 100g mushrooms were added. The containers were then covered and moved in a 30°C environmental chamber for incubation.

2.3.2 Sampling of salt-brined mushrooms

The mushrooms held in the 7% brine were sampled every day for 3 days following equilibration. The mushrooms held in the 14% brine were sampled every 7 d following incubation for 14 days, and the mushrooms held in the 21% brine were sampled every 14 d following incubation for 56 days. Two control and two treatment containers of each NaCl concentration were sampled on each interval. NaCl concentration of the control mushroom brines was monitored and recorded to note changes if any. The brine from each container was decanted into a 15% bleach solution so that only the mushrooms remained in the container to be used for enumeration of *S. aureus* and SE detection.

2.4 Effect of chitin on growth and enterotoxin production of *S. aureus*

For logistical purposes, this experiment was divided into two sections: One examined BHI broth with and without 14% NaCl and blanched mushrooms in 14% NaCl brine, and another examined the effect of 0.5% chitin with or without 14% NaCl, also in BHI broth. Methods used for each section are provided below. Results from these sections were then used to make comparisons between treatments.

2.4.1 BHI broth vs. mushroom experiment

This experiment was done in order to establish how BHI broth, with or without 14% NaCl, performed as a medium for growth and enterotoxin production by *S. aureus*. Dilution bottles were prepared with 100 ml of

either BHI broth or BHI broth + 14 % NaCl and subsequently sterilized. Blanched mushrooms were prepared in 14% NaCl brine as described earlier. The bottles were inoculated with a prepared *S. aureus* culture so that the initial cell concentration was approximately 2×10^3 CFU/ml. The mushrooms were inoculated and subdivided into individual containers as per the same procedure described in the salt-brine experiment. Following inoculation, the bottles and mushrooms were placed in a 30°C environmental chamber for incubation. The BHI broth without NaCl was sampled every 5 hours for a total of 25 hrs while the BHI with 14% NaCl was sampled every day for 8 d after inoculation. The brined mushrooms were sampled on days 0, 4, 6, 8 after inoculation. In all cases, each sampling time involved removing either 3 bottles of each treatment or 3 plastic containers of mushrooms. The bottles were used to make appropriate serial dilutions at each time interval while each container of mushrooms was prepared as discussed in the salt-brined mushroom protocol and subsequently used to make appropriate serial dilutions. As before, *S. aureus* and total aerobic plate count were enumerated on Baird-Parker medium and TSA medium, respectively.

2.4.2 Chitin experiment

In order to examine the effect of chitin on growth and enterotoxin production of *S. aureus* grown under selected condition, BHI broth with and without 14% NaCl added was prepared with 0.0% and 0.5% chitin added during preparation. This concentration was shown to be the optimum concentration of chitin for enterotoxin production from previous work done at by Anderson et al.[6] Additionally, chitosan, a deacetylated form of chitin, was examined at a similar concentration. A sufficient number of dilution bottles were prepared with 100 ml of one of the following: 1). BHI + 0.5% Chitin; 2). BHI + 0.5% Chitosan; 3). BHI + 0.5% Chitin + 14% NaCl; 4). BHI + 0.5% Chitosan + 14 % NaCl. Each bottle was inoculated with a prepared *S. aureus* 743 culture to arrive at an initial cell concentration of ~ 2×10^3 CFU/ml. The bottles were then incubated at 30°C for a time period which allowed for growth and toxin production. The treatments without 14% NaCl were sampled every day for 7 days after inoculation.

Table 1 contains results of analyses of the various BHI broth preparations. Included are salometer readings, salt meter measurements, and A_w values.

Table 1. Analysis of NaCl brines after equilibration with blanched mushrooms

Brine	Salometer	Titration Conc	A_w
7%	27.0	7.4%	0.945
14%	54.0	15.5%	0.892
21%	80.5	23.6%	0.825

All values are means of two samples

During each sampling period, three bottles of each treatment were removed from incubation and were used to make appropriate serial dilutions in peptone dilution blanks. As in previous experiments, *S. aureus* and total plate count were enumerated on Baird-Parker medium and TSA medium, respectively.

2.5 Detection of staphylococcal enterotoxin

After the bacterial enumeration procedures of the mushrooms were completed, 100g samples of the mushroom blend was carefully transferred to sterile centrifuge bottles and centrifuged for 20 min / 4°C / 10,000g. It was assumed that if any staphylococcal enterotoxin was present in the sample blend, it was present in the supernatant. A 2ml aliquot of the supernatant was removed and stored in cryogenic Nalgene tubes at -70°C until needed. Similarly, 2ml aliquots of the BHI broth samples were removed and stored, however the broth cultures were not centrifuged prior to this removal. The miniVIDAS®(bioMerieux Vitek, Inc., Hazelwood, MI) system was used

to detect for staphylococcal enterotoxin in samples. This system is a diagnostic assay system that is used to perform an automated qualitative enzyme-linked fluorescent immunoassay (ELFA) for the detection of staphylococcal enterotoxins in food ingredients and products. The test employs a solid-phase receptacle (SPR) which is coated with anti-enterotoxin antibodies which is used to transfer various reagents along a sealed strip which contains the reagents. The configuration of the miniVIDAS system prevents non-specific reactions with the SPR. All assay steps are performed by the miniVIDAS instrument. A 0.5 ml aliquot of sample was pipetted manually into one of the reagent strips and if enterotoxin was present in the sample, it bound to the anti-enterotoxin polyclonal antibodies in the SPR. Antibodies conjugated with alkaline phosphatase were then cycled in and out of the SPR and will bind to any enterotoxin bound to the SPR wall. The assay utilizes a fluorescent substrate, 4-methyl-umbelliferyl phosphate, which undergoes a conversion into its fluorescent product, 4-methyl-umbelliferone upon catalysis by the alkaline phosphatase bound to enterotoxin. The intensity of fluorescence is related to the amount of SE present in a sample, and is printed as a Test Value (TV) after analysis by the system's on-board computer. Any TV < 0.13 was interpreted as a negative sample while any sample with a TV ≥0.13 was considered positive for staphylococcal enterotoxin. Final SE concentrations were estimated using a standard curve generated using highly purified (96%) staphylococcal enterotoxin A and plotting TV versus known SE concentrations (data not shown).

Table 2. Analysis of BHI broth preparations

Treatment	Salometer	Salt Meter	A_w
BHI broth	7.0	1.10%	0.988
BHI broth + 0.5% chitin	53.0	14.0%	0.905
BHI broth + 14% NaCl	7.0	1.10%	0.979
BHI broth + 0.5% chitin + 14% NaCl	53.0	14.10%	0.900
Distilled water	NR	0.0%	0.994

All values are means of two samples. NR: Not Readable

2.6 Statistical analysis

All statistical analyses were performed using StatView Version 4.5 (Brainpower, Inc., Berkeley, CA). ANOVA tables and Fisher's Protected Mean Separation procedure were used to determine significant differences between temperatures and other treatments in regard to *S. aureus* growth and enterotoxin production. Additionally, StatView was used to calculate standard error of the mean which was used as the basis for Y-error bars found in the graphs of the results section.

3 Results and Discussion

3.1 Growth and enterotoxin production of *S. aureus* in blanched mushrooms held in NaCl brine solutions at 30°C

Both growth of *S. aureus* and enterotoxin production occurred in blanched mushrooms held in both 7% and 14% salt-brines within 14 days at 30°C (Table 3). However, no growth of *S. aureus* or SE production occurred in 21% NaCl brine. Staphylococcal enterotoxin was detected within 2 d of storage in 7 % NaCl and by 7 d in 14%NaCl (Table 3). Although it was suspected that enterotoxin may be produced in 7% NaCl brine, the fact that it occurred after only 2 days was rather surprising. By day 3 of storage, SE concentration in the 7% NaCl had increased to 48.43 ng/ml. Clearly, 7% NaCl failed to prevent growth or production of SE by *S. aureus* in blanched mushrooms held under abusive time/temperature conditions. The results obtained from the study in

14% NaCl differ from those reported by Marland[7] who reported that enterotoxin was not produced at 12% or 14% NaCl in culture broth. This new evidence suggests that higher NaCl concentrations may be required to prevent growth and enterotoxin of *S. aureus* in salt-brined, blanched mushrooms. The blanched mushrooms held in 21% NaCl brine did not support growth or SE production by *S. aureus* within 56 d (Table 3). In fact, at this high salt concentration, none of the initial *S. aureus* inoculum could be detected upon equilibration. Marth [8] reported that in environments with > 20% NaCl, staphylococci survived for only a few days. It did take approximately 2 days for the 21% brine to equilibrate but after that time, no typical *S. aureus* colonies were recovered on the selective Baird-Parker medium.

Table 3. Growth and enterotoxin production by *Staphylococcus aureus* in salt-brined mushrooms

NaCl	Day	Log CFU/g	VIDAS Reading	Estimated SEA(ng/ml)
7%	1	4.55	0.07(-)	0.00
	2	6.98	0.25(+)	7.14
	3	7.79	1.17(+)	48.44
14%	1	4.87	0.06(-)	0.00
	2	8.36	0.60(+)	22.98
	3	7.85	1.16(+)	47.76
21%	*	*	*	*

Each value is a mean of two samples. +/- indicates whether VIDAS reading was considered positive or negative for SE based on a threshold value of 0.13.
* No *S. aureus* growth or enterotoxin was detected in mushrooms held in 21% brine.

Experiments were conducted to evaluate growth of *S. aureus* in 14% salt in mushrooms compared to BHI broth. Results of this experiment demonstrated that growth and SE production by *S. aureus* occurred more rapidly in blanched mushrooms in 14% NaCl brine compared to BHI broth with 14% NaCl (Figure 1). The inhibitory effect of 14% NaCl was more pronounced in BHI broth than in mushrooms (Figure 1). By day 7 of incubation, the *S. aureus* population in BHI broth with 14% NaCl had grown to approximately 10^6 CFU/g, which typically is sufficient for production of enterotoxin, but no enterotoxin was detected at this time. This indicates that, in addition to stimulating the growth of *S. aureus*, SE production is enhanced by mushrooms containing 14% NaCl compared to BHI broth with 14% NaCl. For example, by day 8 of incubation, the *S. aureus* population in the mushrooms had grown beyond 10^7 CFU/g and SE was detected at an average concentration of 6.77 ng/ml (Figure 1).

These results further demonstrate that mushrooms may be more prone to staphylococcal food poisoning than other foods if contaminated with *S. aureus* and held in NaCl brine for an extended period. Within two days, the *S. aureus* population in BHI broth decreased, however growth resumed after 3 days of incubation (Figure 1). This trend is similar to that seen by Helmy et al. [9] who reported that at 15% NaCl, growth of *S. aureus* was repressed during the first 24 hours, but then resumed at a slow rate after an initial lag. The most important observation, however, was that production of SE in mushrooms held in 14% NaCl brine was greater than that in BHI broth with 14% NaCl. Although no significant differences were present, a trend of increased SE production in mushrooms was observed (Figure 1). In any event, this trend of increased SE production, combined with the significant increase in growth of *S. aureus* permitted by the mushrooms, demonstrates that blanched mushrooms in 14% NaCl may be a superior growth medium for *S. aureus*. Overall, in the presence of 14% NaCl, growth of *S. aureus* was significantly more rapid in mushrooms and SE production was suppressed in BHI broth.

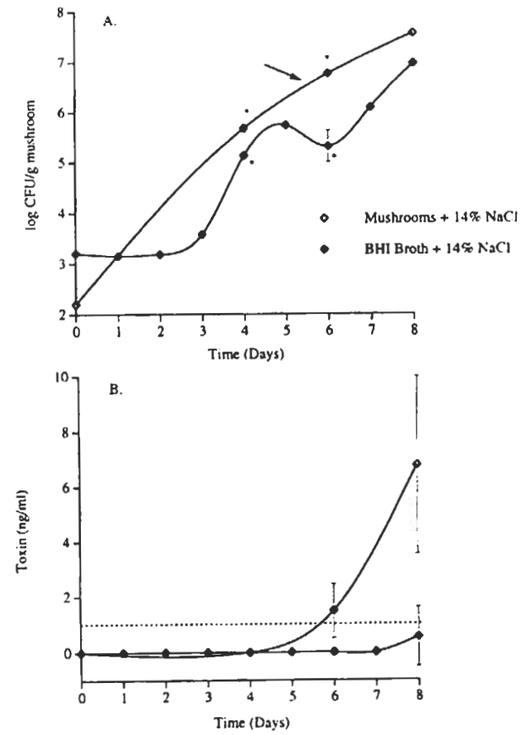

Figure 1. Growth of *S. aureus* 743 (A) and enterotoxin production (B) in blanched mushrooms held in 14% salt brine at 30°C

Data points are means of three samples and error bars represent standard error of the mean. Means followed by an (*) are significantly different ($p < 0.05$). Arrows (A) and where curves cross dashed line (B) represent time at which samples were positive for SE.

3.2 Effect of chitin on growth and enterotoxin production of *S. aureus*

Results from this experiment demonstrated that an increase in growth rate and SE production by *S. aureus* occurred when 0.5% chitin was added to BHI broth (Figure 2). However, the effect of chitin on these parameters was only significant early in the incubation period (Figure 2). For example, growth of *S. aureus* was significantly higher ($p < =0.05$) in the presence of 0.5% chitin only during the logarithmic phase of growth. Also, this increase in growth correlated to significantly higher SE production within the same period. With 0.5% chitin added, SE concentration was significantly higher only at 10 and 15 hr of incubation (Figure 2B). At 15 hr, production of 39.93 ng/ml SE occurred in BHI without chitin while 62.98 ng/ml was produced in the BHI with 0.5% chitin; this represented a 63% increase in enterotoxin production by the BHI containing 0.5% chitin. After 25 hr of incubation, 88.50 ng/ml and 85.08 ng/ml SE was produced in BHI broth with and without 0.5% chitin, respectively; this difference was not significant ($p<=0.05$). Also, no difference was observed in the growth of the *S. aureus* population at this time.

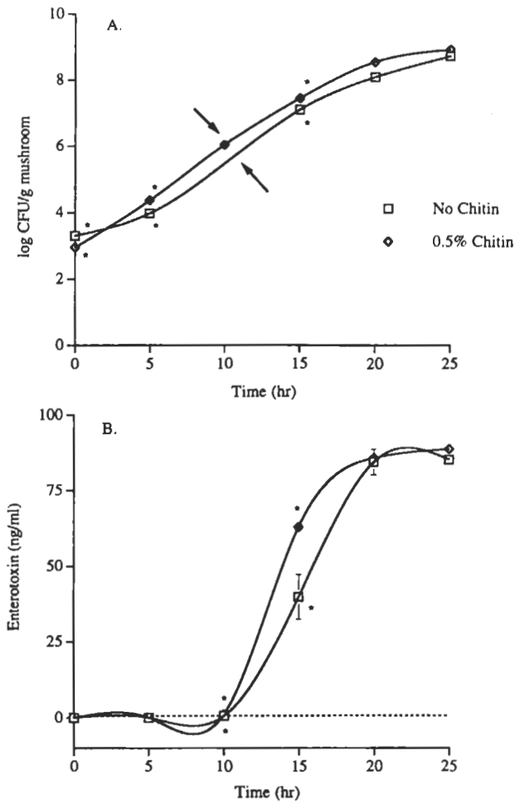

Figure 2. Growth (A) and enterotoxin production (B) by *S. aureus* 743 in BHI broth with and without 0.5% chitin at 30°C

Data points are means of three samples and error bars represent standard error of the mean. Means followed by an (*) are significantly different ($p < 0.05$). Arrows (A) and where curves cross dashed line (B) represent time at which samples were positive for SE.

These results confirm those reported by Anderson et al.[6] They reported that addition of chitin to BHI broth increased SE production by 52-88% without increasing the *S. aureus* population. However, they did not present data regarding when the increase in toxin formation occurred or how growth of *S. aureus* was affected early in the growth cycle. Although this study did not find a significant increase in SE production after 20 hours as reported by Anderson et al.,[6] this difference may be attributed to the methodology used in each study. The previous study used agitation of the BHI broth with chitin with a magnetic stir plate whereas the present study did not. This may account, in part, for the differences observed in SE production. The effect of 0.5% chitin added to BHI broth on growth and SE production of *S. aureus* was more dramatic in the presence of 14% NaCl (Figure 3). The initial population of *S. aureus* in BHI broth with 0.5% chitin was significantly lower than that in BHI without added chitin and decreased during the first day. However, after day 2, growth of *S. aureus* was consistently and significantly higher with 0.5% chitin added, suggesting that chitin may have component(s) which enhance *S. aureus* growth in stressful environments of reduced water activity. In addition to its effect on *S. aureus* growth, 0.5% chitin had a highly significant effect on SE production in the presence of 14% NaCl (Figure 3B.). From the time of inoculation through day 4 of incubation, no SE production occurred with or without chitin. Between days 4 and 5, SE was detected in the BHI broth with 0.5% added chitin and continued to increase through day 7. At that time, 17.15 ng/ml SE was detected with added chitin and no toxin was detected without it.

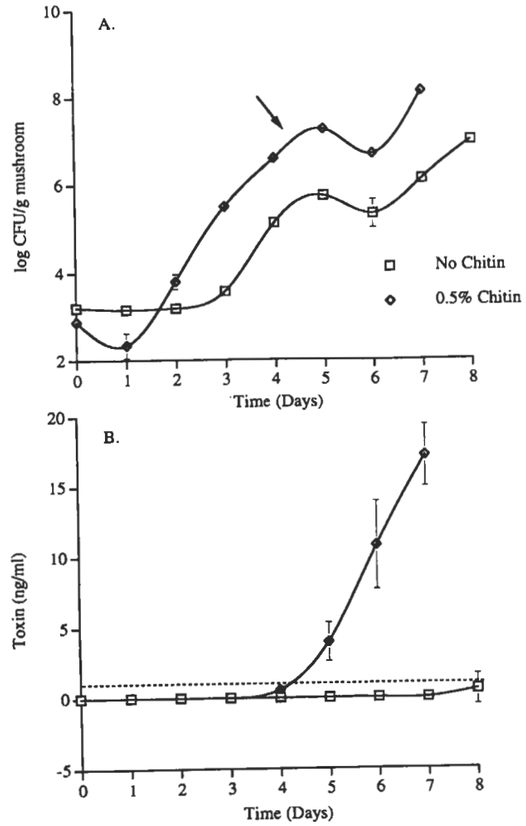

Figure 3. Growth (A) and enterotoxin production (B) by *S. aureus* in BHI broth with 14% NaCl with and without 0.5% chitin at 30°C

Data points are means of three samples and error bars represent standard error of the mean. Means followed by an (*) are significantly different ($p < 0.05$). Arrows (A) and where curves cross dashed line (B) represent time at which samples were positive for SE.

3.3 Effect of chitosan

Chitosan was chosen in order to examine the effect of a chitin-derivative material on growth and SE production of *S. aureus*. Addition of 0.5% chitosan had very similar effects as the 0.5% chitin on growth and SE production of *S. aureus* in BHI broth with and without 14% NaCl (Figures 4 and 5). The only notable exception was that 0.5% chitosan permitted significantly higher SE production that 0.5% chitin after 10 hr of incubation in the absence of 14% NaCl (Fig 4). Results from this set of experiments was interesting in that growth of *S. aureus* was not inhibited by chitosan, as was reported in several previous studies.[10-12] As mentioned earlier, the chitosan used in the present study was in the form of crude flakes, whereas the chitosan used in the earlier studies was solubilized in dilute acid. With both chitin and chitosan bringing about similar effects on growth and SE production by *S. aureus*, perhaps a characteristic common to both is responsible.

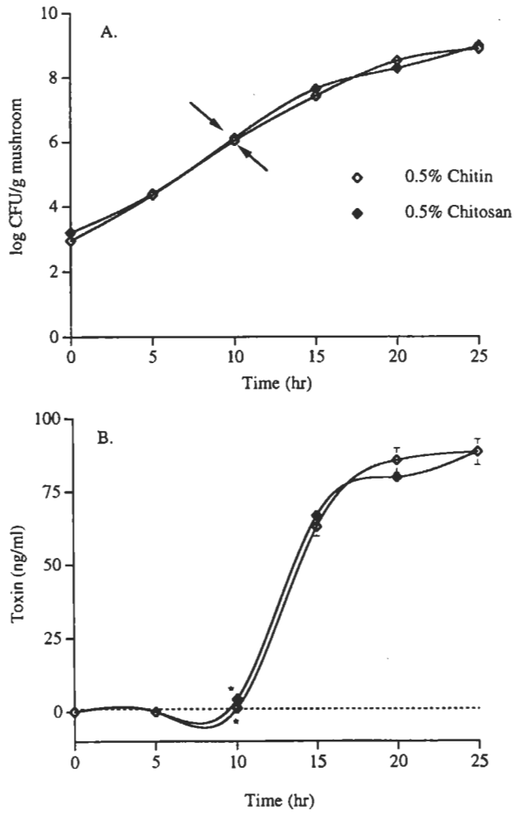

Figure 4. Growth of *S. aureus* 743 (A) and enterotoxin production (B) in BHI broth with 0.5% added chitin or chitosan at 30°C

Data points are means of three replicate experiments and error bars represent standard error of the mean. Means followed by an (*) are significantly different ($p < 0.05$). Arrows (A) and where curves cross dashed line (B) represent time at which samples were positive for SE.

4 Conclusions

Blanched mushrooms held in NaCl brines of 14% or less for extended periods at 30°C supported growth and SE production of *S. aureus*. This could be of practical significance, due to certain practices that may be employed in mushroom factories. In some cases, factories hold blanched mushrooms in weak salt brines for several days when excessive raw product is received that exceeded plant capacity.[2] Also, blanched mushrooms are often held in heavy NaCl brines in China for several months before being de-salted and subsequently thermally processed. Hence, if the NaCl concentration in the product decreases, a dangerous situation could be created. It is suggested that if salt-brining is part of the manufacturing protocol, careful monitoring of the NaCl concentration be mandatory so that high enough NaCl concentration be maintained in order to reduce the risk of staphylococcal food poisoning.

Examination of the increased osmotic stress created by the NaCl may lead to a better understanding of its effect on SE production. 0.5% chitin in BHI broth enhanced SE production during log phase growth of *S. aureus* and early in the growth cycle. Since chitin is found naturally in mushrooms in a similar concentration these results give further evidence that chitin may be responsible for enhanced growth and SE production of *S. aureus*.

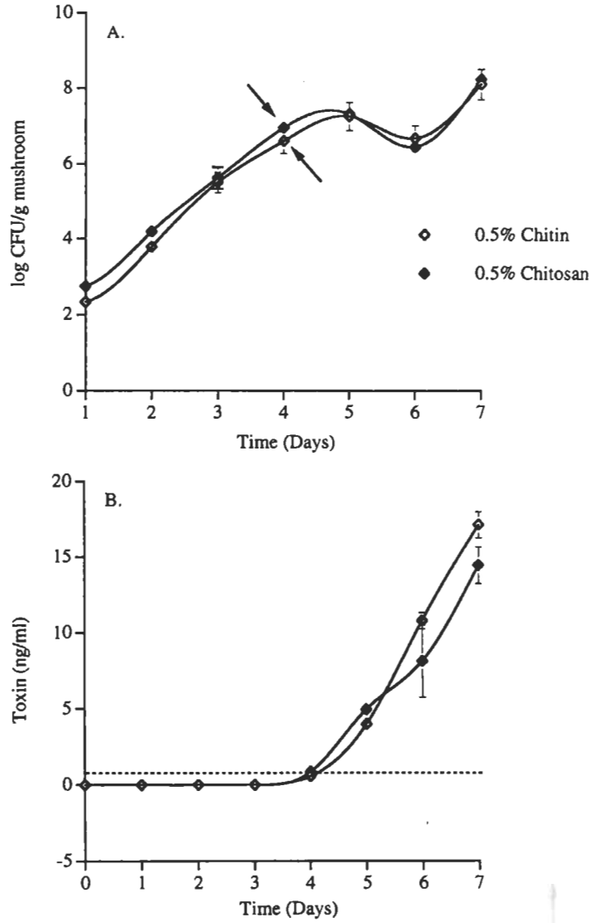

Figure 5. Growth of *S. aureus* 743 (A) and enterotoxin production (B) in BHI broth with 14% NaCl and with 0.5% added chitin or chitosan at 30°C

Data points are means of three replicate experiments and error bars represent standard error of the mean. Means followed by an (*) are significantly different ($p < 0.05$). Arrows (A) and where curves cross dashed line (B) represent time at which samples were positive for SE.

Acknowledgements

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