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Effect of Sodium Chloride on Subsequent Survival of *Staphylococcus aureus* in Various Preservatives

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Abstract

Chemical preservatives in foods are nowadays added at lower concentrations. However, this may allow survival of bacterial cells and induce increased resistance to various preservatives. In this study, the effects of growth in NaCl (10% or 15%) on survival of *Staphylococcus aureus* strains in various chemical and physical preservatives were investigated. Growth of the strains for 20 h at 37˚C in nutrient broth containing 10% NaCl enhanced survival in chemical preservatives (e.g. nutrient broth containing 20% NaCl, or 0.3% thyme extract, or 0.1% ascorbic acid). Growth at 37˚C for 20 h in nutrient broth containing 15% NaCl or for 5 d in nutrient broth containing 10% NaCl greatly enhanced survival of the strains in the tested preservatives. For survival at low temperature (5˚C) (physical preservative), cells grown at 37˚C for 20 h in nutrient broth containing 10% NaCl were not more tolerant to low temperature. Growth of the strains at 37˚C for 20 h in nutrient broth containing 15% NaCl or for 5 d in nutrient broth containing 10% NaCl only slightly increased the survival of cells at low temperature.

Keywords

NaCl, *Staphylococcus aureus*, Preservatives

1. Introduction

Staphylococcus spp., especially *S. aureus*, is the second most common cause of food borne diseases worldwide [1] [2]. It has been reported that in USA, for example, the estimated number of cases of staphylococcal gastroenteritis is 1 - 2 million per year [3]. The symptoms of staphylococcal gastroenteritis usually develop after 1 - 6 hours after ingestion of contaminated food, and include nausea, vomiting, diarrhea and abdominal cramps. About 10% of the affected persons may be admitted to hospital but the mortality rate is very low or nil [3]. Staphylococcal gastroenteritis results from consumption

of one or more preformed heat-resistant enterotoxins that are produced in food contaminated with *staphylococcus* [4]. The amount of enterotoxin necessary to cause gastroenteritis in humans is very small. It has been reported that, in an outbreak of staphylococcal food poisoning due to chocolate milk, the mean amount of staphylococcal enterotoxin A in a 400-ml container was 144 ng [5].

S. aureus is relatively resistant to drying and high temperature (it withstands 50˚C for 30 min). It grows well in media containing 9% NaCl, and some strains can grow in media containing higher concentrations of sodium chloride [6]. Food borne outbreaks of *S. aureus* are usually associated with food products that are made by hand and not properly refrigerated after preparation. The most important vehicle foods include meat, chicken, milk, and their products [1].

Chemical preservatives are usually added to prevent spoilage of food and extend its shelf life. Because consumers prefer minimally processed foods, chemical preservatives are nowadays added at lower concentrations. However, some bacterial cells may survive in the food and this stress may induce increased resistance to various conditions. To my knowledge, there are no studies on the response of *S. aureus* to these conditions. Therefore, this study was conducted to investigate the effects of growth of *S. aureus* strains under suboptimal conditions (e.g. growth in medium containing moderate amount of sodium chloride) on their survival in some chemical and physical preservatives.

2. Materials and Methods

2.1. Bacterial Strains

Staphylococcus aureus strains used in this study were previously isolated by the author. The strains were isolated from raw milk from dairy processing plant, and from raw milk from dairy farm.

2.2. Media and Chemicals

Crude extract of *Thymus vulgaris* (extracted by hydrodistillation) was obtained from Systema Co. Ltd. (Amman, Jordan). Stock solution of ascorbic acid was freshly prepared before each use and sterilized by filtration through membrane filters (0.45 um, Micron Separation Inc., Philadelphia, Pa., USA).

2.3. Effect of NaCl on Survival of the Strains

A 0.5 ml of overnight grown bacterial culture was added to tubes containing 10 ml nutrient broth plus 10% NaCl or nutrient broth plus 15% NaCl. Inoculated tubes were incubated static at 37˚C for 20h or 5 days. Then, 0.5 ml of the appropriate culture was added to flasks containing 30 ml nutrient broth plus 20% NaCl, nutrient broth plus 0.3% *Thymus vulgaris* extract, or nutrient broth plus 0.1% ascorbic acid; and flasks were incubated static at 37˚C for 4 days. For survival at low temperature, 0.5 ml of the above mentioned bacterial cultures (*i.e.* bacterial cultures grown in nutrient broth plus 10% NaCl or in nutrient broth plus 15% NaCl) were added to flasks containing 30 ml of nutrient broth alone and incubated at 5˚C for 12 days. At appropriate times, bacterial

cultures (0.1 ml) were diluted in 0.85% NaCl and number of *S. aureus* cells was determined by plating on nutrient agar and incubation of plates at 37˚C for 48 h.

For control experiments, 0.5 ml of the overnight grown bacterial culture was directly added to flasks containing 30 ml nutrient broth plus 20% NaCl, nutrient broth plus 0.3% *Thymus vulgaris* extract, or nutrient broth plus 0.1% ascorbic acid; and flasks were incubated static at 37˚C for 4 days. For growth at low temperature (control experiment), 0.5 ml of the overnight grown bacterial culture was directly added to flask containing 30 ml nutrient broth alone and incubated at 5˚C for 12 days. Number of viable cells was enumerated as mentioned above.

2.4. Statistical Analysis

All experiments were done four times with consistent results. The log number of cells presented are the mean values and the standard error for these readings ranged from ±0.1 to ±0.2. Student's *t*-test was used to determine the significant differences ($p < 0.05$) among the different culture conditions.

3. Results

The isolated strains were tested; and the results shown are of representative strain from raw milk from dairy processing plant (*S. aureus* 5) and of representative strain from raw milk from dairy farm (*S. aureus* 16).

3.1. Growth of *S. aureus* at Various Conditions (Control Experiments)

The effect of various preservatives on *S. aureus* 5 and *S. aureus* 16 grown for 20 h in nutrient broth alone was examined (Table 1). Presence of 20% NaCl in the medium

Table 1. Effect of incubation for 20 h in nutrient broth alone on survival of *S. aureus* 5 (a) and *S. aureus* 16 (b) in nutrient broth at various conditions.

(a)

Growth conditions	Log CFU/ml		% increase (decrease) in log CFU/ml[c]
	t_0	t	
NB + 20% NaCl, 37˚C	7.0	6.6[a]	(6)
NB + 0.3% thyme extract, 37˚C	6.5	6.7[a]	3
NB + 0.1% ascorbic acid, 37˚C	6.5	7.2[a]	10
NB, 5˚C	7.0	6.1[b]	(13)

(b)

Growth conditions	Log CFU/ml		% increase (decrease) in log CFU/ml[c]
	t_0	t	
NB + 20% NaCl, 37˚C	6.0	4.6[a]	(23)
NB + 0.3% thyme extract, 37˚C	6.1	6.8[a]	11
NB + 0.1% ascorbic acid, 37˚C	6.1	7.4[a]	21
NB, 5˚C	6.2	5.5[b]	(11)

[a]Growth after 4 days. [b]Growth after 12 days. [c]% increase (decrease) in log CFU/ml = 100× (log CFU/ml at the specified time-log CFU/ml at time zero/log CFU/ml at time zero).

slightly decreased growth of *S. aureus* 5, where the percentage of decrease in log number of cells after 4d incubation was 6% (Table 1(a)). The presence of 0.3% *T. vulgaris* extract or 0.1% ascorbic acid in the medium slightly promoted growth of *S. aureus* 5. The percentages of increase in log number of cells after 4d incubation were 3% and 10%, respectively.

Nearly similar results were obtained for *S. aureus* 16 (Table 1(b)). Presence of 20% NaCl in the medium decreased growth of this strain, but the percentage of decrease was more than that of *S. aureus* 5. The presence of 0.3% *T. vulgaris* extract or 0.1% ascorbic acid in the medium slightly promoted growth of *S. aureus* 16. The percentages of increase in log number of cells after 4d incubation were 11% and 21%, respectively (Table 1(b)).

The effect of low temperature on growth of the tested *S. aureus* strains in nutrient broth alone is shown in Table 1. For both strains, the number of cells did not change after 8 days of incubation (data not shown), but slightly decreased after 12 d incubation at 5˚C.

3.2. Effect of Growth in NaCl on Survival of *S. aureus* in Chemical Preservatives

The effect of growth for 20 h in nutrient broth containing 10% NaCl on survival of *S. aureus* strains at various conditions is presented in Table 2. Both strains showed a great increase in levels of survival at the tested conditions, but the percentage of increase in survival varied among the strains. *S. aureus* 5 showed greater increase in survival than *S. aureus* 16. *S. aureus* 5 cells grown for 20 h in nutrient broth containing 10% sodium chloride significantly survived more in nutrient broth plus 20% NaCl, nutrient broth plus 0.3% thyme extract or nutrient broth plus 0.1% ascorbic acid ($p < 0.05$). The percentages of increase in log cell number after 4 d incubation were 19%, 40% and 48%, respectively (Table 2(a)). This represents 25, 13 and 4.8 fold, respectively, increase in log cell number compared to the survival of *S. aureus* 5 cells grown in nutrient broth alone and then subjected to these preservatives (Table 1(a) & Table 2(a)). For *S. aureus* 16 treated under the same conditions, the percentages of increase (decrease) in log number of cells after 4 d incubation in nutrient broth plus 20% NaCl, nutrient broth plus 0.3% thyme extract or nutrient broth plus 0.1% ascorbic acid were (10%), 34% and 40%, respectively. This represents 2.3, 3 and 2 fold, respectively, increase in log cell number compared to the survival of *S. aureus* 16 cells grown in nutrient broth alone and subjected to these preservatives ($p < 0.05$) (Table 1(b) & Table 2(b)).

Effect of growth for 20 h in nutrient broth containing 15% NaCl on survival of *S. aureus* 5 is presented in Table 3(a). Incubation of the tested strain under this condition also increased ($p < 0.05$) the survival of the cells in nutrient broth plus 20% NaCl, nutrient broth plus 0.3% thyme extract or nutrient broth plus 0.1% ascorbic acid (Table 3). The percentages of increase in log cell number after 4 d incubation were 29%, 33% and 36%, respectively (Table 3(a)). For *S. aureus* 16 cells grown under the same conditions, nearly similar results were observed. The percentages of increase in log cell

Table 2. Effect of incubation for 20 h in NB containing 10% NaCl on survival of (a) *S. aureus* 5 and (b) *S. aureus* 16.

(a)

Growth conditions	Incubation time (d)	Log CFU/ml	% increase (decrease) in log CFU/ml
NB + 20% NaCl, 37˚C	0	5.7	-
	1	6.9	21
	4	6.8	19
NB + 0.3% thyme extract, 37˚C	0	5.3	-
	1	7.7	45
	4	7.4	40
NB + 0.1% ascorbic acid, 37˚C	0	5.2	-
	1	7.6	46
	4	7.7	48
NB, 5˚C	0	6.1	-
	12	4.5	(26)

(b)

Growth conditions	Incubation time (d)	Log CFU/ml	% increase (decrease) in log CFU/ml
NB + 20% NaCl, 37˚C	0	5.8	-
	4	5.2	(10)
NB + 0.3% thyme extract, 37˚C	0	5.3	-
	4	7.1	34
NB + 0.1% ascorbic acid, 37˚C	0	5.2	-
	4	7.3	40
NB, 5˚C	0	6.0	-
	12	5.1	(15)

number after 4 d incubation in nutrient broth plus 20% NaCl, nutrient broth plus 0.3% thyme extract or nutrient broth plus 0.1% ascorbic acid were 0%, 30% and 34%, respectively, ($p < 0.05$) (Table 3(b)).

Increasing the incubation period of *S. aureus* in nutrient broth containing 10% sodium chloride to 5 days further increased the survival of the tested strains in nutrient broth plus 20% NaCl, nutrient broth plus 0.3% thyme extract or nutrient broth plus 0.1% ascorbic acid ($p < 0.05$). For *S. aureus* 5, the percentages of increase in log cell number after 4 d incubation in these preservatives were 30%, 56% and 67%, respectively (Table 4(a)). This represents 36, 19 and 6.7 fold, respectively, increase in log cell number compared to the survival of *S. aureus* 5 cells grown in nutrient broth alone and subjected to these preservatives (Table 1(a) & Table 4(a)). Also, similar results were obtained for *S. aureus* 16. The percentages of increase in log cell number after 4 d incubation in nutrient broth plus 20% NaCl, nutrient broth plus 0.3% thyme extract or

Table 3. Effect of incubation for 20 h in NB containing 15% NaCl on survival of (a) *S. aureus* 5 and (b) *S. aureus* 16.

(a)

Growth conditions	Incubation time (d)	Log CFU/ml	% increase (decrease) in log CFU/ml
NB + 20% NaCl, 37˚C	0	5.5	-
	1	5.9	7
	4	7.1	29
NB + 0.3% thyme extract, 37˚C	0	5.4	-
	1	7.8	44
	4	7.2	33
NB + 0.1% ascorbic acid, 37˚C	0	5.3	-
	1	7.4	40
	4	7.2	36
NB, 5˚C	0	5.2	-
	12	4.8	(8)

(b)

Growth conditions	Incubation time (d)	Log CFU/ml	% increase (decrease) in log CFU/ml
NB + 20% NaCl, 37˚C	0	5.6	-
	4	5.6	0
NB + 0.3% thyme extract, 37˚C	0	5.4	-
	4	7.0	30
NB + 0.1% ascorbic acid, 37˚C	0	5.3	-
	4	7.1	34
NB, 5˚C	0	5.5	-
	12	5.0	(9)

nutrient broth plus 0.1% ascorbic acid were 4%, 42% and 54%, respectively (Table 4(b)).

3.3. Effect of Growth in NaCl on Survival of *S. aureus* at Low Temperature

S. aureus 5 and *S. aureus* 16 cells grown for 20 h in nutrient broth containing 10% NaCl did not survive more in nutrient broth at low temperature (5˚C) (Table 2). However, growth of the tested strains for 20 h in nutrient broth containing 15% NaCl or for 5 d in nutrient broth containing 10% NaCl slightly increased ($p < 0.05$) the survival of *S. aureus* 5 and *S. aureus* 16 cells in nutrient broth at low temperature (Table 3 & Table 4).

4. Discussion

S. aureus can be found in raw milk, on surfaces of fresh meat, poultry and fish, and on skin of humans. Chemical preservatives are usually added to inhibit growth of bacteria

Table 4. Effect of incubation for 5 days in NB containing 10% NaCl on survival of (a) *S. aureus* 5 and (b) *S. aureus* 16.

(a)

Growth conditions	Incubation time (d)	Log CFU/ml	% increase (decrease) in log CFU/ml
NB + 20% NaCl, 37˚C	0	6.3	-
	1	7.6	20
	4	8.2	30
NB + 0.3% thyme extract, 37˚C	0	5.9	-
	1	8.4	42
	4	9.2	56
NB + 0.1% ascorbic acid, 37˚C	0	5.8	-
	1	8.3	43
	4	9.7	67
NB, 5˚C	0	6.1	-
	12	5.5	(10)

(b)

Growth conditions	Incubation time (d)	Log CFU/ml	% increase (decrease) in log CFU/ml
NB + 20% NaCl, 37˚C	0	6.4	-
	4	6.7	4
NB + 0.3% thyme extract, 37˚C	0	6.1	-
	4	8.7	42
NB + 0.1% ascorbic acid, 37˚C	0	6.1	-
	4	9.4	54
NB, 5˚C	0	5.9	-
	12	5.5	(7)

and extend shelf life of foods. However, food and food products are sometimes minimally processed, which may allow survival of bacterial cells and induce increased resistance to various preservatives.

4.1. Effect of Growth in NaCl on Survival of *S. aureus* in Chemical Preservatives

In this study, growth of *S. aureus* strains for 20 h in nutrient broth containing 10% or 15% NaCl increased the subsequent survival of the strains in nutrient broth containing various preservatives (e.g. 20% NaCl, 0.3% thyme extract, or 0.1% ascorbic acid). These findings are consistent with other studies on *Salmonella* spp. and *E. coli*. It has been reported that *E. coli* O157:H7 cells that were adapted to acid (by culturing for one to two doublings at pH 5) had an increased resistance to lactic acid and survived better in shredded dry salami (pH 5) and apple cider (pH 3.4) [7]. Also, in *Salmonella typhimurium*, acid adaptation induced cross-protection against environmental stresses such as

heat, salt and surface-active agents [8], and in *E. coli* K12, prior exposure to acidic conditions increased survival of cells at low pH [9].

Mild stresses can trigger a general stress response system in the bacterial cell causing increased resistance to preservatives and environmental factors. The Rpo S sigma factor is the regulator of the general stress in *E. coli* and *Salmonella* spp. when acid shock or starvation are encountered [10]. In *S. aureus*, a similar stress response may be triggered alone or in combination with other systems.

In this study, the tested strains showed increased survival in various preservatives after growth in 10% or 15% NaCl, but the level of increase was greater in *S. aureus* 5 than in *S. aureus* 16. *S. aureus* 16 was isolated from raw milk from dairy farm while the former strain was isolated from raw milk from dairy processing plant, and may be therefore exposed to other stresses (e.g. long time at high temperature) before arrival to the processing plant. Similar findings were reported by Abu-Ghazaleh *et al.* [11], and Abu-Ghazaleh [12] who reported that genetic and environmental factors influence the tolerance of food poisoning bacteria to heat and chemical preservatives. Also, Leyer *et al.* [7] emphasized the need to take into account the previous history of strains used in food challenge studies which could affect their survival in the environment.

4.2. Effect of Growth in NaCl on Survival of *S. aureus* at Low Temperature

In this study, growth of the tested strains in NaCl (10% or 15%) did not increase or slightly increased survival of the strains at low temperature (5˚C). Studies on other types of bacteria (e.g. *E. coli* O157:H7) have showed that exposure of the strain to low pH (such as beef gravy medium containing 5% NaCl, pH 4.5), enhanced survival of cells at 7˚C compared to 22˚C [13]. However, the present study examined survival of *S. aureus* strains at lower temperature (5˚C). Also, *S. aureus* may be less tolerant to low temperature than *E. coli*.

5. Conclusion

This study has showed that growth of *S. aureus* strains at suboptimal conditions (e.g. 10% or 15% NaCl) enhances their survival in chemical preservatives. This suggests that although minimal processing may increase the shelf life of foods, it causes concerns in some foods that contain food poisoning bacteria, such as *S. aureus*, that can adapt to suboptimal growth conditions and survive in these foods.

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