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Absence or presence of glucose in growth medium and its effect on heat injury in *Staphylococcus aureus*

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SUMMARY

Staphylococcus aureus 196E, when grown in a glucose ($\geq$0.25% wt./vol.)-containing medium, produced cells that would undergo injury when subjected to sublethal heat conditions (45 min at 50°C); however, if glucose was omitted from the growth medium, the extent of injury was greatly reduced. Media containing glucose sterilized by filtration or by separate autoclaving produced cells equal in injury susceptibility to medium in which glucose was autoclaved as part of the medium components. Injury also occurred when other sugars such as fructose, mannose, maltose, or lactose were substituted for glucose. Sugar-containing media that produced *Staphylococcus aureus* of maximal susceptibility to heat injury reached a pH of approximately 6 or lower during growth of the cells. Incubation of staphylococci in growth medium acidified with acetic or lactic acids or HCl did not lead to cells that would undergo injury under the stated conditions. The stimulatory effect of glucose on injury appears to be related to the metabolism of the sugar by *Staphylococcus aureus*.

INTRODUCTION

Previous investigators of heat injury in *Staphylococcus aureus* predominantly employed either BBL's trypticase soy broth [1,2,6,7] or Difco's tryptic soy broth [3,4,8,10] for culturing the organism. These products each contain 0.25% glucose. During a series of studies on heat injury in *S. aureus* 196E, we inadvertently used tryptic soy broth without glucose (Difco; TSB w/o glucose) as the growth medium to prepare cells for injury. The amount of heat injury (45 min at 50 C) obtained from cells grown on TSB w/o glucose was considerably less than that obtained from regular TSB which contains 0.25% glucose. In this paper, we report the effect of growing *S. aureus* 196E in the presence of glucose and other sugars on heat injury.

METHODS AND MATERIALS

Preparation of cells

S. aureus 196E was inoculated into 100 ml tryp-

* Agricultural Research Service, U.S. Department of Agriculture. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

tic soy broth (Difco; TSB) or tryptic soy broth w/o glucose (Difco; TSB w/o glucose) and incubated on a rotary shaker (200 rpm) at 37°C for 16 h. Contents of the culture flasks were centrifuged at 16 000 × *g* for 5 min at 5°C, washed three times with sterile potassium phosphate buffer (0.1 M; pH 7.2), and resuspended in 3 ml sterile distilled water. In some experiments, desired concentrations of carbohydrates were added to TSB w/o glucose and the broth-carbohydrate mixtures were sterilized by autoclaving.

Heat injury and assay for injured cells

Washed cells were added to sterile potassium phosphate buffer (pH 7.2, 0.1 M) to give approximately $5 \cdot 10^9$ cells/ml and were heated at 50°C for 45 min [10]. Aliquots were removed from the injury flasks and dilutions were prepared in 0.1% peptone (Difco) water blanks. Appropriate dilutions were surface plated on tryptic soy agar (Difco) plus 1% pyruvate and on tryptic soy agar plus 7% NaCl using a spiral plater (Spiral Systems Instruments, Incl., Bethesda, MD). Plates were counted after 2 days incubation at 37°C. Tryptic soy agar plus pyruvate permits growth of both injured (injured cells can repair on the medium before starting to grow) and noninjured cells. The salt-containing agar only allows growth of noninjured cells because heat-injured *S. aureus* lose their salt tolerance and cannot repair the injury in the presence of high concentrations of salt [11].

RESULTS AND DISCUSSION

The data presented in Fig. 1 indicate that there was more than a 600-fold increase in injury when cells were grown in regular TSB as compared to cells grown in TSB w/o glucose. Similar results were obtained when the growth medium was trypticase soy broth with or without glucose (BBL; Fig. 1). A different production lot of TSB w/o glucose gave similar results. Thus, the results suggest that the presence of glucose in the growth medium potentiated heat injury in *S. aureus*.

The increase in injury observed with cells grown

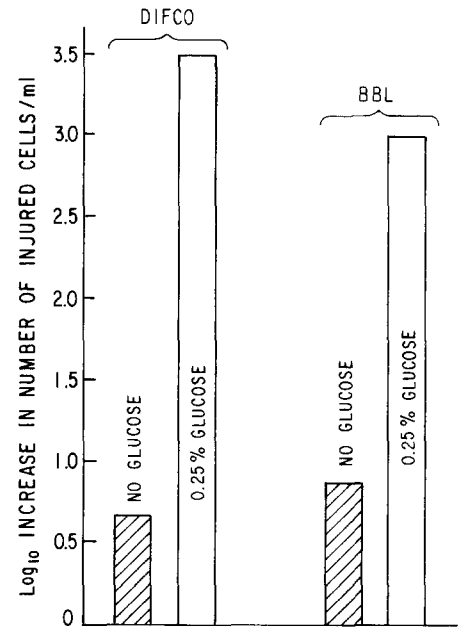

Fig. 1. The effect of absence or presence of glucose in the growth medium (Difco's tryptic soy broth ± glucose; BBL's trypticase soy broth ± glucose) on heat injury in *S. aureus* 196E.

in the presence of 0.0625, 0.125, 0.25, 0.5, or 1.0% glucose was 1.26, 2.43, 3.0, 2.82, and 3.03 log units, respectively. Thus, the maximum stimulation of injury was obtained when *S. aureus* was grown in the presence of at least 0.25% glucose. In the experiments described in Fig. 1, glucose was autoclaved with the medium. Sugars heated in the presence of amino acids produce microbial growth inhibitory compounds [5], which suggests that glucose autoclaved with proteinaceous materials may affect the cells so that they are more liable to heat injury. However, when media containing glucose added after separate autoclaving or after filter sterilization (0.45 micron Nalgene Sterilization Filter Unit, type S) were employed, cells had similar susceptibility to heat injury as cells grown in media containing glucose autoclaved in the medium (Fig. 2).

Potentiation of heat injury was also observed when other carbohydrates were substituted for glucose in the growth medium (Table 1). The most effective compounds were fructose, maltose, glucose, lactose and mannose. Glycerol, arabinose and xylose had little potentiating effect on heat injury. All

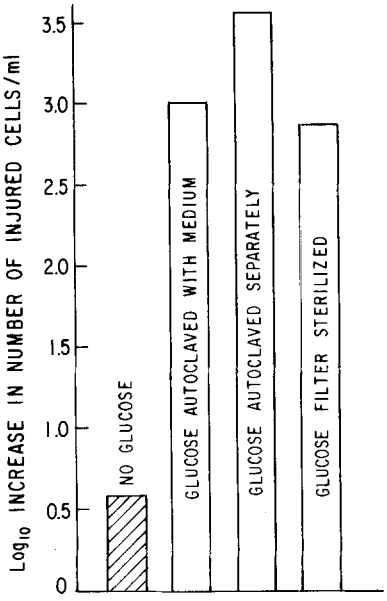

Fig. 2. Effect of sterilization method of glucose (added to TSB w/o glucose) on heat injury in *S. aureus* 196E.

Table 1

The effect of carbohydrate addition to growth medium on heat injury to *S. aureus* 196E

Addition[a]	Log_{10} increase in number of injured cells/ml	pH at the end of growth (16 h)[b]
None	0.44	7.1
Fructose	3.34	6.1
Maltose · H_2O	3.26	5.5
Glucose	3.00	5.9
Lactose	2.84	5.1
Mannose	2.73	6.3
Sucrose	1.83	5.4
Mannitol	1.77	6.1
Glycerol	0.69	6.7
Arabinose	0.61	6.8
Xylose	0.27	6.8

[a] Basal medium was TSB w/o glucose (Difco); all carbohydrates were added at 13.9 mM (equivalent to 0.25% glucose). Carbohydrates were autoclaved with the medium.

[b] pH measurements were made on culture media utilizing an Orion Research pH meter, model 601 A equipped with Fisher pencil combination electrode, model E-5M.

of the compounds tested except arabinose and xylose are metabolized by *S. aureus* 196E [9]. The data presented in Table 1 suggest also that stimulation of heat injury by carbohydrate incorporation into the growth medium is related to the pH of the medium at the end of growth ($\leqslant$pH 6.1).

The data presented in Table 2 describe studies on the effect of pH of the growth medium on the potentiation of heat injury in *S. aureus*. Staphylococci were grown in TSB w/o glucose $\pm$0.25% glucose for 16 h (designated 0 h) and then the media were either acidified or alkalinized. The media (and cells) were then incubated for an additional 2.5 h

Table 2

The effect of pH of the growth medium and pH adjustment after growth on heat injury of washed cells of *S. aureus* 196E

S. aureus was grown in the described media for 16 h and the pH determined (called 0 h); additions were made and flasks were incubated for an additional 2.5 h when the pH was determined again (called 2.5 h). Cells were then harvested, washed, and subjected to injury. Numbers in parentheses represent pH values before addition of acid or base.

	Growth medium	pH 0 h	pH 2.5 h	Log_{10} increase in number of injured cells/ml
1.	TSB w/o glucose	7.6	7.6	0.60
2.	TSB w/o glucose + 0.25% glucose	6.3	6.3	3.38
3.	same as 1 but pH adjusted to 6.3 by addition of HCl at 0 h	6.3 (7.6)	6.3	0.96
4.	same as 1 but pH adjusted to 6.3 by addition of acetic acid at 0 h	6.3 (7.5)	6.2	0.60
5.	same as 1 but pH adjusted to 6.3 by addition of lactic acid at 0 h	6.3 (7.5)	6.3	0.41
6.	same as 1 but 0.25% glucose added at 0 h	7.5	6.4	2.70
7.	same as 2 but pH adjusted to 7.6 with NaOH at 0 h	7.6 (6.3)	7.5	2.97

before the cells were harvested, washed and subjected to stress conditions. Acidification of the TSB w/o glucose medium after 16 h growth did not lead to potentiation of injury (3, 4 and 5 in Table 2) after 2.5 h further incubation, whereas addition of 0.25% glucose (6) followed by further incubation did lead to a decrease in pH of the medium and substantial injury to the cells. Metabolic action on glucose by *S. aureus*, as indicated by a decrease in pH, led to injury, whereas direct addition of acid did not.

When the acid produced by growth of *S. aureus* in glucose-containing medium (2 in Table 2) was neutralized by the addition of NaOH (7), only a slight decrease in injury was noted (compare injury obtained from cells grown in 2 and 7). Thus, neutralization of the acid environment produced by growth of *S. aureus* in the presence of glucose did not eliminate the potentiating effect on injury given by glucose. The results obtained in Table 2 suggest that a decrease in pH per se is not responsible for the injurious effects noted when *S. aureus* is grown in the presence of glucose; the decrease in pH is merely indicative of glucose metabolism.

The basis for the glucose effect on heat injury is not readily apparent. The effect appears to be related to *S. aureus* metabolism of glucose.

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