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Prevention of *Staphylococcus aureus* Lysis

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Staphylococcus aureus S-6 cells grown in chemically defined media often lysed after exponential growth. Lysis could be prevented by the addition of alanine or proline before the culture reached stationary phase.

Chemically defined media for *Staphylococcus aureus* S-6 have been described by Mah et al. (2), Wu and Bergdoll (8), and Keller et al. (1). It was observed that with the medium used by Keller et al. (1), most *S. aureus* S-6 cells lysed after exponential growth. This report indicates how alterations in medium components can prevent *S. aureus* cell lysis after exponential growth.

S. aureus S-6 (a gift from M. S. Bergdoll, Food Research Institute, University of Wisconsin, Madison, Wis.), which is widely used for studying enterotoxin B synthesis, was used in this study. Storage of the culture, preparation of inocula, and incubation conditions have been described previously (6).

Growth of *S. aureus* S-6 in both the chemically defined medium of Keller et al. (1) and brain heart infusion (BHI) (Difco Laboratories, Detroit, Mich.) is shown in Fig. 1. When 18 amino acids (final concentration, 50 μg/ml) were added to the growth medium (1), cell lysis was observed after the exponential growth phase, as evidenced by a decrease in measured turbidity (Fig. 1) and by microscopic observation. The addition of glucose (0.2%) to the chemically defined medium resulted in similar growth and lysis (data not shown). Cells grown in BHI did not lyse during the same time period. Doubling the concentration of each amino acid of chemically defined media to 100 μg/ml did not prevent cell lysis. The lysis of postexponential cells in chemically defined medium did not appear to be due to bacteriophage, since plaque assays (7) of

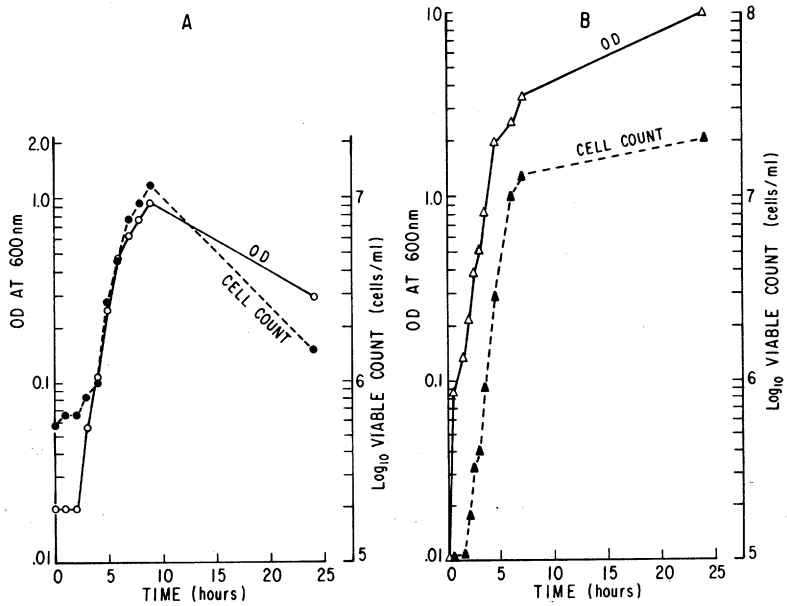

FIG. 1. Comparison of *S. aureus* S-6 growth in chemically defined medium and BHI. Growth was assayed by turbidity measurement at 600 nm. Cell viability was determined on BHI-spread plates counted after 2 days of incubation at 37°C. (A) Chemically defined medium; (B) BHI. The data presented here are the results of one of several experiments that all showed similar responses. OD, Optical density.

TABLE 1. Effect of postexponential media supplementation on *S. aureus* S-6 lysis[a]

Addition to growth medium[b]	% of protection against lysis[c]
None	25
BHI (0.6%)	84
Salt mix (2× concn)	64
Amino acid mix	100
Amino acid mix without:	
Lysine	100
Threonine	100
Serine	100
Glycine	100
Alanine	35
Proline	46

[a] Data presented here are the results of one of several experiments that all showed similar responses.

[b] Salt mix (2× concentration) as described in the synthetic medium by Keller et al. (1). Amino acid mix contained lysine, threonine, serine, glutamic acid, glycine, alanine, and proline, each at a concentration of 1.2 mg/ml.

[c] Cell lysis was monitored by turbidity measurement at 600 nm. Percentage of protection against lysis = absorbance at the end of the exponential growth phase × 100/absorbance at 24 h.

lysed cultures of *S. aureus* RN450 (a gift from J. J. Iandolo, Division of Biology, Kansas State University, Manhattan, Kans.) were negative.

To further investigate cell lysis, the effect of adding specific amino acids, salt mixture, or BHI to the chemically defined media after the cells reached the exponential growth phase was studied. The addition of high concentrations of specific amino acids to chemically defined medium has been shown to enhance the growth of the cells (1, 2, 6). Based on these reports, a mixture of seven amino acids, including lysine, threonine, serine, glutamic acid, glycine, alanine, and proline, each at a final concentration of 1.2 mg/ml, was added after the exponential growth phase, when the absorbance (600 nm) of the culture medium was ca. 1.0 (Fig. 1). The addition of the seven amino acids to the medium completely eliminated the decreased absorbance caused by cell lysis when the absorbance of the control culture decreased to 25% of the original level (Table 1). Cultures which did not lyse remained 100% viable for at least 24 h, as determined by viable plate counts. The addition of BHI (0.6%) or double the salt mix (1) to the medium after cells reached the exponential phase also partially prevented lysis (Table 1). The omission of lysine, threonine, serine, glutamic acid, or glycine from the amino acid mixture did not affect lysis; however, the omission of alanine or proline did produce cell lysis (Table 1). The optimum concentration of alanine for prevention of lysis was 240 μg/ml, with

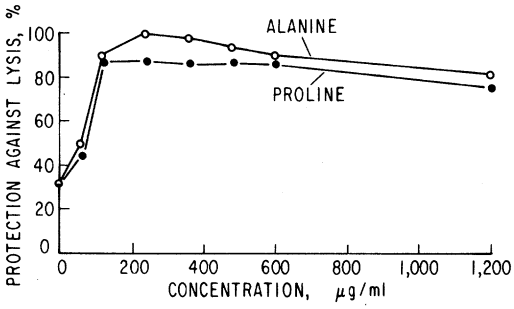

FIG. 2. Effect of alanine and proline concentration on protection against lysis of *S. aureus* S-6. Cell growth and lysis were monitored as described in footnote c of Table 1. The data presented here are the results of one of several experiments that all showed similar responses.

increased lysis occurring at higher alanine concentrations (Fig. 2). A similar response was observed when proline at various concentrations was added to the medium. However, even at its optimum concentration, proline did not completely prevent lysis. When a different strain of *S. aureus* (196E) was used, alanine also prevented cell lysis during postexponential growth, suggesting that the need for alanine is not strain specific.

These results suggest that the lysis of postexponential cells is due to depletion of alanine or proline. Other investigators have demonstrated that bacterial cell wall synthesis can continue after exponential growth and that rapid lysis can occur when an amino acid that is a major component of cell wall (e.g., glycine, glutamic acid, lysine, or alanine) is exhausted from the growth medium (3–5). This could account for the prevention of lysis and the extension of stationary phase viability by the addition of alanine. However, this would not account for the ability of proline to partially prevent lysis in the absence of alanine. Identification of the role of proline will require further study. Likewise, the mechanism by which increasing the salt mixture concentration partially prevents lysis will require additional investigation.

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