Loss of the Penicillinase Plasmid After Treatment of *Staphylococcus aureus* with Sodium Dodecyl Sulfate

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Two strains of *Staphylococcus aureus* (PC1 and 196E), when grown in medium containing 0.002% sodium dodecyl sulfate (SDS), showed from 96.1 to 100% loss of the ability to produce penicillinase. Resistance to cadmium and zinc was lost concomitantly with the ability to produce penicillinase. A comparison of the rate of curing by SDS with the curing effects exerted by elevated temperature and ethidium bromide suggested that SDS is a more effective plasmid curing agent for susceptible strains of *S. aureus* than methods in use at present.

It has been well established that the genes controlling the synthesis of penicillinase in most strains of *Staphylococcus aureus* (and possibly in *S. epidermidis* as well) are borne on a plasmid (1, 2, 6, 10). Barber (2) observed that penicillinase production was spontaneously and irreversibly lost at a low frequency, and it was subsequently reported that an increased frequency of loss resulted after growth of some strains at elevated temperature (9), or after exposure to compounds which interfere with deoxyribonucleic acid (DNA) replication such as acridine dyes (6, 7) and ethidium bromide (4). This paper describes our observation of very high rates of elimination of the plasmid coding for penicillinase production from two strains of *S. aureus* (PC1 and 196E) by growth in the presence of the anionic surface-active agent sodium dodecyl sulfate (SDS).

**MATERIALS AND METHODS**

**Strains and media.** Thirty-eight strains of *S. aureus*, all of which produced penicillinase, were employed in this investigation. The two most frequently used were 196E, a constitutive immunological type C, received from M. H. Richmond, and PC1 (13), a constitutive immunological type A received from S. Cohen. Cultures were maintained on yeast extract-Trypticase soy (YETS) slants which contained Trypticase Soy Broth (BBL), 0.3% yeast extract (Difco), and 1.5% agar (Difco).

**Curing of the plasmid state.** Small inocula (10^4 cells per ml) of penicillinase-positive (P+) strains were incubated with constant shaking at 37 C for 18 hr in YETS broth containing 0.002% SDS (Matheson, Coleman and Bell, Norwood, Ohio). Colonies arising from cells which had lost the penicillinase plasmid were detected by the method of Baldwin et al. (1).

**RESULTS**

Growth in YETS broth containing 0.002% SDS resulted in elimination of the penicillinase plasmid from both *S. aureus* 196E and *S. aureus* PC1, although the kinetics of growth and curing were different for each strain. Figure 1A shows the number of P+ and penicillinase-nonproducing (P-) cells as a function of time in a culture of *S. aureus* 196E growing in YETS broth containing 0.002% SDS. The inoculum was adjusted to yield 5.5 x 10^7 P+ and fewer than 5 x 10^6 P- cells/ml. After 1 hr, the population contained 4.7 x 10^6 cells/ml, of which 3.3 x 10^4 were P-. After the initial sharp decrease in number of viable cells, the growth rate increased and was logarithmic by the third hour of incubation. The residual number of P+ cells in the culture decreased to 10^5/ml and remained at that level for the remainder of the incubation period. A separate control culture of YETS broth containing 0.002% SDS was inoculated with cells from a colony of 196E which had spontaneously lost its penicillinase plasmid, thereby becoming P-. The kinetics of growth in the P- control culture indicated that growth of P- cells could not have accounted for the sharp initial increase in the number of P- cells seen during growth in SDS. The P- control culture increased in number from 8.7 x 10^4 to 1.3 x 10^6 cells/ml during the first hour of incubation, whereas the number of P- cells in the P+ inoc-
ulum increased from fewer than $5 \times 10^8$ cells/ml to $3.3 \times 10^9$ cells/ml during the same time period.

Figure 1B shows the total number of cells and the number of $P^-$ cells as a function of time in a culture of $S. aureus$ PC1 growing in YETS broth containing 0.002% SDS. There was no decrease in total number as with $S. aureus$ 196E. The total cell number steadily increased throughout the incubation period, but the number of $P^-$ cells increased at a much greater rate. After 4 hr of incubation, the $P^-$ cells comprised over 80% of the total viable cells. A $P^-$ control inoculum of $1.8 \times 10^8$ cells/ml in YETS broth containing 0.002% SDS showed only a slight rise in cell number after incubation for 3 hr, to $3.7 \times 10^8$ cells/ml. This result, which was comparable to the behavior of the $P^-$ culture of $S. aureus$ 196E, indicated that growth of the relatively small number of $P^-$ cells spontaneously arising in $P^+$ cultures of the two strains would not have accounted for the increase in $P^-$ cells seen during growth in YETS containing SDS.

No growth was observed when PC1 and 196E were incubated in YETS broth containing an SDS concentration of 0.003% or greater. After growth in YETS broth containing 0.001% SDS or less, the percentage of $P^-$ colonies was equal to the control values for both strains.

It has been shown that the genetic material responsible, in $S. aureus$, for resistance to cadmium and zinc is also present on the plasmid containing the markers for penicillinase production (12). These properties should, therefore, be expected to be eliminated by SDS curing along with penicillinase production. To test this hypothesis, colonies derived from cells grown in YETS with and without SDS, plated on YETS agar, were replica-plated on YETS agar containing either $5 \times 10^{-4}$ M CdCl$_2$ or $3 \times 10^{-3}$ M Zn(SO$_4$)$_2$. No growth of colonies of SDS-treated cells was noted on the replica plate, whereas 97 to 99% of the colonies from a control culture grew. It was assumed that colonies which failed to replicate arose from cells which spontaneously lost their plasmids.

Susceptibility to curing does not appear widespread. Thirty-eight $P^+$ strains of $S. aureus$ were grown in YETS containing SDS at concentrations slightly less than the minimal inhibitory concentration for each strain to test for plasmid elimination. Of the 38 tested, only two strains ($S. aureus$ 196E and $S. aureus$ PC1) showed significant increases in the number of $P^-$ cells. Included in the strains tested for curing were strains U9 and 8325 $m^+ P^-$ which have been shown to contain a plasmid-borne penicillinase gene (6, 11). Both of these strains showed no increased frequency of $P^-$ cells as a result of treatment with SDS.
The relative rates of plasmid elimination by SDS, ethidium bromide, and growth at elevated temperature (43 to 44 C) were compared. The concentration of ethidium bromide giving the highest percentage of P- cells from P+ cultures of S. aureus 196E was $3 \times 10^{-6}$ M. These results (Table 1) suggest that SDS is a much more effective plasmid curing agent for this strain than either ethidium bromide (26.2%) or elevated temperature (16.7%).

**DISCUSSION**

Various methods of eliminating the penicillinase plasmid from S. aureus have been reported (4, 6, 7, 9). These methods effect curing either by taking advantage of differences in the rates of plasmid and chromosomal DNA replication during growth at elevated temperature (9) or by the use of agents such as the acridine dyes (6, 7) and ethidium bromide (4) which are believed to inhibit the synthesis of DNA. The rates of curing with SDS that we observed far surpassed the rates obtained with ethidium bromide and elevated temperature (Table 1). Rates of plasmid elimination achieved with ethidium bromide vary from 8 to 100% (4) and with acridine dyes from 0.1 to 3.9% (3, 7). Growth at 43 to 44 C has been reported to yield from 0.1 to 58% loss of the ability to produce penicillinase (1, 9). Growth in SDS results in elimination of the entire plasmid, as evidenced by the simultaneous loss of penicillin, cadmium, and zinc resistance.

Since SDS is known to cause the disruption of biological membranes, the possibility exists that SDS is curing the penicillinase plasmid by disrupting the membrane sites of plasmid attachment. Very little is known of the intracellular localization and the mechanism for replication and maintenance of the penicillinase plasmid. Novick and Richmond (11) have proposed a membrane attachment site and a plasmid-borne maintenance-compatibility locus which may govern its attachment and replication. Elimination of the penicillinase plasmid by SDS may lend further support to a plasmid-membrane association which, when disrupted, leads to loss of the plasmid. The fact that only two of 38 P+ strains tested were cured by growth in SDS may indicate that the membrane attachment site of the plasmid in these two strains is more susceptible to the effects of SDS.

Curing of the sex (F) factor in *Escherichia coli* by use of SDS was reported by Inuzuka and co-workers (8), but these authors were not sure whether SDS was exerting a selective pressure on the F- cells or was actually curing the plasmid by disrupting the intracellular membrane binding site of the F particle. Recently, Dulaney tested over 12,500 compounds in an effort to obtain compounds capable of eliminating the penicillinase plasmid from S. aureus (5). He was specifically interested in compounds which were relatively nontoxic and effective in very low concentration for possible use as agents to make penicillin-resistant *S. aureus* infections susceptible to penicillin treatment. Several compounds were reported to kill penicillinase plasmid-bearing cells selectively, but none was capable of plasmid elimination. Our evidence does not conclusively indicate a disruption of membrane attachment sites during growth in SDS, but does seem to rule out a selection of P- cells. It appears to us that SDS may be a useful agent in the study of extrachromosomal genetic elements and their relationship to the cell membrane.

**ACKNOWLEDGMENTS**

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**Table 1. Comparison of plasmid elimination from *S. aureus* 196E by SDS, ethidium bromide, and elevated temperature**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Total colonies</th>
<th>P- colonies</th>
<th>Percent elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>1,239</td>
<td>39</td>
<td>3.1</td>
</tr>
<tr>
<td>SDS (0.002%)</td>
<td>926</td>
<td>926</td>
<td>100.0</td>
</tr>
<tr>
<td>Ethidium bromide</td>
<td>431</td>
<td>113</td>
<td>26.2</td>
</tr>
<tr>
<td>Elevated temperature</td>
<td>203</td>
<td>34</td>
<td>16.7</td>
</tr>
</tbody>
</table>

*Concentration giving highest rate of plasmid elimination was $3.0 \times 10^{-6}$ M.

*Elevated temperature experiments were conducted at 43 to 44 C.

*Control culture was incubated at 37 C with constant shaking.

**LITERATURE CITED**


