ANTIBIOTIC RESISTANCE AND ACID PRODUCTION AMONG STARTER CULTURES BELONGING TO THE GENUS STREPTOCOCCUS

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The susceptibility to penicillin of a wide range of single strain cultures of lactic streptococci was determined. Approximately 50% of the strains studied exhibited resistance to penicillin at the 0.1 unit/ml level. Eight of these cultures were adapted to grow in the presence of 0.5 unit penicillin/ml, but they were unable to produce acid at a rate or quantity equal to that of their sensitive parent strains. Cultures obtained from naturally resistant isolates produced more acid in the presence of low penicillin concentrations than their sensitive parent counterparts, but less than that produced by "trained" resistant strains from the same parent origin.

The presence of low levels of antibiotics in the milk supply has posed many problems for the dairy industry. From the manufacturer's viewpoint, the decreased acid production by starter cultures as a result of antibiotics is of great economic significance. Suggestions such as the use of penicillinase, excessively large quantities of starters, or antibiotic-resistant starter cultures have been offered as means to avoid or overcome slow down in acid production or complete starter failure.

Much of the work of previous investigators on the effect of antibiotics on starter organisms was done with commercial mixed cultures, of which the strain composition was unknown. However, investigations with Streptococcus cremoris, Streptococcus lactis, Streptococcus durans, Streptococcus diacetilactis and Streptococcus thermophilus (2, 4, 5, 6) have indicated that penicillin concentrations between 0.05 and 0.5 unit/ml milk are sufficient to reduce acid production by these cultures to a degree that would impair the cheesemaking process.

The objectives of the study reported herein were: (a) to determine the resistance to penicillin of a wide range of single strain cultures of lactic streptococci; (b) to increase, by a training program, the resistance of those cultures which tolerated low levels of penicillin; and (c) to isolate naturally resistant cells from selected cultures in order to attempt to obtain satisfactory acid producers.

ExPERIMENTAL RESULTS

Survey of lactic streptococci for penicillin resistance

A total of 35 strains of lactic acid producing-streptococci, including 18 strains of S. cremoris, 13 strains of S. lactis, 2 strains of S. diacetilactis and 2 strains S. thermophilus obtained from culture collections in the United States, New Zealand and Australia, were screened for susceptibility to low levels of penicillin. The criterion of susceptibility was the amount of acid produced in sterile skim milk containing 0.1 unit penicillin/ml compared to that developed by the same culture in plain skim milk. Incubation was at the rate of 1% with a 16 hour culture. Incubation was at 30°C with titratable acidity determined after 2, 4, 6, 8, 12 and 24 hours. Nineteen of the cultures were unable to produce appreciable amounts of acid under the stated conditions. The remaining 16 cultures produced titratable acidity values of 0.28 to 0.74% in eight hours. Although this was usually somewhat less than the control culture, it did indicate some measure of resistance to penicillin.

The 16 cultures which grew in the presence of 0.1 unit penicillin/ml were subjected to further studies to determine their resistance to a range of penicillin concentrations from 0.1 to 1.0 unit/ml. Results, representative of five of these cultures (3 strains S. lactis and 2 strains S. cremoris), presented in Table 1, indicate a high degree of resistance to 0.1 unit penicillin/ml. After six hours incubation, the decrease in acid production was only from 0.1 to 0.05% for four cultures while acid produced by culture S. cremoris US was decreased by 0.12%. After 24 hours incubation, the titratable acidity values were 0.05 to 0.12% less than the controls.

None of the cultures produced satisfactory acid levels in the presence of 0.25 unit or more of penicillin/ml. The levels of acid produced after eight hours of incubation in milk containing 0.25 unit/ml were 0.32 — 0.48% less than the controls. Titratable acidity values after 24 hours incubation revealed that culture S. cremoris US was completely inhibited by 0.5 unit penicillin/ml. Acid production by all cultures was completely inhibited by 1.0 unit penicillin/ml. Results obtained with the remaining 11 cultures (6 strains of S. lactis and 5 strains of S. cremoris) were in agreement with those presented in Table 1.

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DEVELOPMENT OF PENICILLIN RESISTANCE

Attempts were made to increase the level of penicillin resistance of the cultures which had demonstrated some degree of natural resistance. The cultures were subjected to a “training program” which consisted of repeatedly transferring them into milks containing a low level of penicillin. The first transfer was into milk to which was added 0.1 unit penicillin/ml. After each culture clotted, it was transferred to milk containing a slightly higher penicillin concentration. This process was repeated until the cultures consistently were able to produce sufficient acid, in the presence of 0.5 unit penicillin/ml, to clot the milk. This “training” required approximately 10 transfers and was successful with eight of the 16 cultures.

Subsequently, titratable acidity produced by these cultures and their sensitive parent strains was determined after 8 and 24 hours incubation. Values typical of those obtained on several occasions are presented in Table 2. These data demonstrate that none of the resistant cultures obtained by the training process were able to produce as much acid as their parent strains. The difference between resistant and sensitive counterparts was very marked at eight hours incubation but became less by 24 hours incubation. Culture S. lactis C**, resistant, produced more acid than any of the other cultures in eight hours; i.e., the titratable acidity produced by it was only 0.18% less than that by the parent strain. The decrease in acid developed by the other seven resistant strains, compared to their parent sensitive strains, was 0.25 to 0.57%.

Because the resistant strain of S. lactis C** was able to produce appreciable acid when grown in the presence of 0.5 unit penicillin/ml, comparisons were made between this culture and its parent strain when grown in milk containing penicillin concentrations up to 1.0 unit/ml. Representative results of these studies, presented in Table 3, illustrate that in the absence of penicillin, the tolerant strain did not produce acid as rapidly as the sensitive strain, and the total acid produced was somewhat less. For example, after eight hours incubation the titratable acidity of the sensitive parent culture was 0.66% compared to 0.54% by the penicillin tolerant culture. This trend was maintained at each sampling time. In the presence of penicillin levels greater than 0.1 unit/ml, the tolerant culture produced appreciably more acid than the sensitive culture, i.e., 0.50% titratable acidity, compared to 0.22% after eight hours incubation in the presence of 0.25 unit penicillin/ml. A penicillin concentration of 1.0 unit/ml prevented acid production by the tolerant culture comparable to that of the sensitive culture at 0.25 unit/ml.

** Isolation and activity of naturally resistant mutants

The 16 cultures originally selected, on the basis of their ability to produce acid in the presence of 0.1 unit penicillin/ml, were assumed to contain some cells which possessed a high level of penicillin resistance, although the population as a whole was not highly resistant. Therefore, attempts were made to isolate these penicillin resistant component cells and, after propagation of the cultures so obtained, to determine their ability to produce acid in the presence of penicillin.

Two procedures were used to make these isolations. The first employed Plate Count agar, to which was added from 0.1 to 10.0 units penicillin/ml. This was

### Table 1—Effect of Penicillin on Acid Production by S. lactis and S. cremoris.

<table>
<thead>
<tr>
<th>Hours incubation</th>
<th>Penicillin concentration (U/ml)</th>
<th>Percent titratable acidity S. lactis</th>
<th>Percent titratable acidity S. cremoris</th>
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<tbody>
<tr>
<td></td>
<td>H1</td>
<td>S**</td>
<td>924</td>
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<td>.73</td>
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<td>.41</td>
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<td>.21</td>
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<tr>
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<td>.20</td>
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<td>.21</td>
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<td>.92</td>
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<td>.48</td>
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<tr>
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<td>.18</td>
<td>.20</td>
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</tbody>
</table>

### Table 2—Acid Production by Cultures of S. cremoris and S. lactis Tolerant of 0.5 Unit Penicillin Per ml.

<table>
<thead>
<tr>
<th>Culture</th>
<th>8 hours incubation</th>
<th>24 hours incubation</th>
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<tbody>
<tr>
<td></td>
<td>Percent titratable acidity</td>
<td>Percent titratable acidity</td>
</tr>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant (trained)</td>
</tr>
<tr>
<td>S. lactis 924</td>
<td>.73</td>
<td>.41</td>
</tr>
<tr>
<td>S. lactis C**</td>
<td>.75</td>
<td>.57</td>
</tr>
<tr>
<td>S. lactis H1</td>
<td>.74</td>
<td>.38</td>
</tr>
<tr>
<td>S. lactis H-1-R</td>
<td>.77</td>
<td>.20</td>
</tr>
<tr>
<td>S. lactis F1K</td>
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<td>.27</td>
</tr>
<tr>
<td>S. lactis T13b</td>
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<td>.33</td>
</tr>
<tr>
<td>S. cremoris 806</td>
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<tr>
<td>S. cremoris US3</td>
<td>.66</td>
<td>.27</td>
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seeded at the rate of 1 ml. of a 10⁻¹ dilution of a 16
hour old culture per 10 ml agar. The plates so pre­
pared were incubated at 30°C for 48 hours. The
colonies which grew were subcultured in sterile skim­
milk. By the second approach, Plate Count agar con­
pared were incubated at 30°C for 48 hours. The
isolates were subcultured three or four times
in sterile skim milk after which acid production was
compared with that of their sensitive and “trained”
resistant counterparts. Titratable acidity determina­
tions were made after 8 and 24 hours incubation at
30°C in the presence of graded levels of penicillin up
to 1.0 unit/ml. Similar results were obtained with
each of the five culture series. The results obtained
with S. lactis C° were typical and are presented in
Table 4. These data reveal that, in the absence of
penicillin, both the sensitive and naturally resistant
strains were capable of greater acid production than
the resistant culture obtained by the training pro­
cess. However, in the presence of penicillin con­
centrations of 0.25 and 0.5 unit/ml, the adapted re­
sistant culture produced appreciably more acid than
its counterparts. After eight hours incubation in milk
containing 0.25 unit/ml, the adapted resistant strain
produced titratable acidity of 0.50% compared to
0.29% and 0.22% by naturally resistant and sensitive
strains respectively. A similar trend was observed
after 24 hours incubation in the presence of both
0.25 and 0.5 unit penicillin/ml.

DISCUSSION

Lactic acid producing bacteria of significance to
the dairy industry reportedly vary widely in their
ability to produce acid in the presence of residual
quantities of antibiotics (8, 10). Results with 35 sin­
gle strain cultures employed in the present study
demonstrated that low levels of penicillin in milk
retard acid production, but that an appreciable por­
tion of starters are able to produce some acid in the
presence of such penicillin concentrations.

The possibility of employing antibiotic-resistant
starters in commercial operations has been sug­
gested, but several investigators have reported (1, 9)
that such cultures do not produce acid at a rate equal to
that produced by sensitive cultures nor suitable for manu­
facturing purposes. The large collection of cultures
employed in the present study, and the results ob­
tained with cultures which were trained to tolerate
penicillin, or which were obtained from isolated
naturally resistant cells, are added evidence to sub­
stantiate the previous views that resistant cultures
do not provide the solution to the problem of slow

TABLE 3—Comparison of Acid Production by Penicillin
Sensitive and Penicillin Tolerant S. lactis C°

<table>
<thead>
<tr>
<th>Hours incubation</th>
<th>Penicillin concentration (U/ml)</th>
<th>Percent titratable acidity</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Sensitive</td>
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<tr>
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<td>.20</td>
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<tr>
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<td>1.00</td>
<td>.18</td>
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<tr>
<td>8</td>
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<tr>
<td>24</td>
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</tbody>
</table>

TABLE 4—Comparison of Acid Production by Penicillin
Sensitive, Naturally Resistant and “Trained” Resistant
Strains of S. lactis C°

<table>
<thead>
<tr>
<th>Hours incubation</th>
<th>Penicillin concentration (U/ml)</th>
<th>Percent titratable acidity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitive</td>
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<tr>
<td>8</td>
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</table>
vats or complete starter failures due to the presence of penicillin.

The assumption that cultures which grow in the presence of low levels of penicillin probably contain some cells which are resistant to higher levels appears invalid in the present study. This is suggested by the fact that the naturally resistant cultures produced no more acid in the presence of 0.5 unit penicillin/ml than their sensitive parent cultures. However, it has been established well (1, 7) that acid production is not a true measure of the ability of lactic acid bacteria to tolerate antibiotics or to multiply in the presence of such compounds. Apparently, antibiotic resistant starters may multiply at a normal rate, but in the process of development of resistance, their ability to convert carbohydrate to lactic acid is altered.

ACKNOWLEDGEMENT

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REFERENCES