Biological Response of Lactic Streptococci and Lactobacilli to Catalase

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Addition of catalase to milk cultures of lactic streptococci resulted in increased rates of acid production, although it had no effect on cultures of lactobacilli. Milk cultures of both streptococci and lactobacilli produced detectable amounts of peroxide, which reached a maximum level in the early period of acid production followed by a drastic decrease as the acid production increased. Pyruvate and reduced glutathione decreased the amount of peroxide formed, but had little effect on acid production by the streptococci. Ferrous sulfate prevented the accumulation of peroxide and stimulated the rate of acid production by the streptococci to a greater extent than did catalase.

Members of the family Lactobacillaceae generally do not produce catalase, although some have been reported to possess enzymatic activity similar to that of catalase or peroxidase when grown under specific cultural conditions (4, 11, 17). Hydrogen peroxide is a metabolic by-product of some species of the Lactobacillaceae (2, 5, 8, 13, 16, 17); however, little is known concerning its effect on cultures producing it. Hydrogen peroxide has been implicated as an autoinhibitor for pneumococci (12), and exogenously added catalase has been reported to stimulate the metabolic activity of several catalase-negative organisms other than the lactic streptococci (3, 11, 16). Several investigators have indicated that certain streptococci and lactobacilli produce detectable levels of hydrogen peroxide (2, 5, 8, 16). On the other hand, some reports state that the peroxide does not accumulate in the growth medium (4, 13). Whether these organisms produce detectable peroxide may depend on the growth medium; also, its detection is dependent on the sensitivity of the assay. Although little attention has been directed toward the autoinhibitory effect of the peroxide, the addition of trace amounts to milk has a deleterious effect on the rate of acid production by lactic streptococci (14).

Since the Lactobacillaceae do not normally produce catalase and have been reported to produce hydrogen peroxide under certain conditions, the possibility exists that levels produced may be autoinhibitory. The present study was undertaken in an effort to establish whether the lactic streptococci and lactobacilli produce autoinhibitory levels of hydrogen peroxide when grown in milk.

MATERIALS AND METHODS

Culture maintenance. All cultures were maintained in sterile litmus milk and were subcultured weekly by using a 1% inoculum and by incubating for 16 to 18 hr. The streptococci were incubated at 22 C, and the lactobacilli were incubated at 35 C. All cultures were held at 3 C between transfers. At least two daily subcultures in litmus milk were made for all cultures before use in an assay.

Acid production. Nonfat milk solids reconstituted at 11% solids and steamed for 30 min were used as the assay medium. The assay medium was inoculated at the desired incubation temperature with 1% of the test culture. The inoculated medium was dispensed in 180-ml volumes into sterile 250-ml Erlenmeyer flasks containing 20 ml of sterile water for the control or 20 ml of filter-sterilized catalase. The catalase (3,000 units/mg; Nutritional Biochemicals Corp., Cleveland, Ohio) was used at a final concentration of 0.01 mg per ml of milk. The inoculated samples were then incubated in a water bath (32 C for the streptococci and 45 C for the lactobacilli). The pH of the cultures was monitored continuously with a pH meter equipped with two automatic electrode switches which permitted the use of 12 combination electrodes connected to a 12-point recorder.

Peroxide measurement. Portions (10 ml) of the control and each sample were removed after various incubation periods to be analyzed for peroxide by the method of Gilliland (7).
RESULTS

The addition of filter-sterilized catalase to the streptococci resulted in more rapid acid production, whereas the lactobacilli exhibited no response (Table 1). The Streptococcus cremoris and S. lactis cultures grown in milk containing catalase produced acid, in an amount sufficient to reach a pH of 5.0, from 21 to 54 min faster than did the control cultures. The commercial cheddar cheese starters exhibited a much wider range of stimulatory response (33 to 126 min). Although there was variation among the cultures, the slower cultures were stimulated more by the catalase than were the faster ones. The Lactobacillus acidophilus and L. bulgaricus cultures were not affected by the addition of catalase (Table 1). Catalase inactivated by autoclaving had no effect on the cultures.

S. lactis AC1 was chosen for further study of the stimulation resulting from exogenously added catalase (Fig. 1). Stimulation of acid production was more prominent during the period of rapid acid production. The peroxide in the control appeared to reach a peak after 3 hr of incubation, followed by a marked decrease and complete disappearance after 6 hr. There was no buildup of peroxide in the culture to which catalase was added. Apparently, the amount of residual peroxide (approximately 1 μg/ml) which accumulated during the early period of incubation of the control culture was sufficient to depress the acid-producing activity of the culture during the period of more rapid acid production.

To study the effect of oxygen on the amount of peroxide produced by S. lactis AC1, a similar experiment was conducted in which air was continuously bubbled through the milk during the incubation period (Fig. 2). The peroxide accumulation in the control culture did not reach a peak in the early stage of acid production, as was observed with the static culture in Fig. 1, but continued to increase during the period of more rapid acid production. Peroxide did not accumulate in the presence of catalase, and acid was produced faster than in control cultures although the rate was less than in the static culture.

L. bulgaricus NYL1 which had been isolated from yogurt was studied similarly. It produced much more peroxide than the streptococci (Fig. 3); however, the prevention of peroxide accumulation by catalase had no effect on acid production. The accumulated peroxide in the control reached a maximum during the early period of incubation followed by a rapid decrease, as was observed with the streptococci.

The effect of pyruvate, reduced glutathione, and ferrous sulfate on acid and peroxide production was measured in cultures of S. lactis AC1 (Fig. 4). Pyruvate reduced the amount of accumu-

### Table 1. Acid production in milk by lactic streptococci and lactobacilli in the presence of exogenous catalase

<table>
<thead>
<tr>
<th>Culture</th>
<th>Time (min) to reach pH 5.0</th>
<th>Amt of stimulation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Catalase</td>
</tr>
<tr>
<td>S. lactis AC1</td>
<td>402</td>
<td>348</td>
</tr>
<tr>
<td>S. lactis AC2</td>
<td>396</td>
<td>345</td>
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<td>S. cremoris KH</td>
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<td>288</td>
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<tr>
<td>S. cremoris ML1</td>
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<td>297</td>
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<tr>
<td>Cheddar cheese starter</td>
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<td></td>
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<tr>
<td>A</td>
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<td>291</td>
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<tr>
<td>Cheddar cheese starter</td>
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<td></td>
</tr>
<tr>
<td>B</td>
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<td>357</td>
</tr>
<tr>
<td>Cheddar cheese starter</td>
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<td></td>
</tr>
<tr>
<td>C</td>
<td>318</td>
<td>285</td>
</tr>
<tr>
<td>L. acidophilus LA-10-65</td>
<td>360</td>
<td>360</td>
</tr>
<tr>
<td>L. bulgaricus NYL1</td>
<td>285</td>
<td>285</td>
</tr>
</tbody>
</table>

![Fig. 1. Acid and peroxide production in milk by S. lactis AC1 as affected by added catalase.](http://aem.asm.org/)  

![Fig. 2. Effect of aeration on acid and peroxide production by S. lactis AC1 in milk with and without catalase.](http://aem.asm.org/)
lated peroxide in the culture, but had very little, if any, effect on the acid production (Fig. 4). Reduced glutathione was more effective than pyruvate in preventing the accumulation of peroxide; however, its slight stimulation of acid production by the culture (as shown in Fig. 4) was irregular in replicate experiments. Peroxide accumulation was prevented when 0.5 mm ferrous sulfate was added to the milk, and the rate of acid production was greatly increased (Fig. 4). Stimulation effected by the ferrous sulfate was greater than that resulting from the addition of catalase.

**DISCUSSION**

Certain members of the family Lactobacillaceae have been reported to produce catalase or "catalase like" enzymes (4, 5, 10, 17); however, this is apparently not a common characteristic and occurs only rarely. For this type of enzymatic activity to be present, the organism must be propagated under specific growth conditions, usually with low levels of fermentable carbohydrates (10). It is not likely that such enzymatic activity would appear in milk cultures of lactic streptococci or lactobacilli. Therefore, if these organisms do produce sufficient peroxide in milk to be self-inhibitory, exogenously added catalase could conceivably result in stimulation of growth.

Our results indicate that the lactic streptococci produce sufficient peroxide to be autoinhibitory. Subramanian and Olson (14) reported that milk to which low levels of hydrogen peroxide (1 to 11 μg/ml) had been added just before inoculation was inhibitory to lactic streptococci. The amounts of peroxide detected in the milk cultures of streptococci in the present study (approximately 1 μg/ml) probably represent only a fraction of the total amount produced by the cultures, since peroxide at low concentrations (1 to 300 μg/ml) is quite unstable in milk (7, 14). Although the lactobacilli included in this study apparently produced more peroxide than the streptococci, it did not have any adverse effect on acid production by the cultures. Wheater et al. (16) indicated that catalase was stimulatory to lactobacilli when grown on glucose. The lack of agreement between our findings and those of Wheater et al. may be due to the difference in test cultures or in the growth medium.

The peroxide produced by static cultures of streptococci and lactobacilli in the present study appeared to accumulate to a maximal level in the early period of acid production, followed by rapid dissipation as the acid production increased. The dissipation of the peroxide during the period of most rapid acid production may have been due to reactions of the peroxide with other metabolic end products or intermediates, such as keto acids or compounds containing free sulf-hydryl groups. We have shown that compounds such as pyruvate and reduced glutathione, when added to milk cultures of S. lactis, prevent or
decrease greatly the accumulation of peroxide. The lack of a marked stimulatory response to these two compounds may have been due to the slower destruction of peroxide by these compounds than by catalase or to the reaction product possibly being slightly toxic to the organism; either one could counteract the favorable effect of destroying the peroxide.

The lack of continued peroxide accumulation in the static culture may have been caused by a limited oxygen supply in the system coupled with the aforementioned mechanisms of dissipation. On the other hand, peroxide in the aerated culture of *S. lactis* AC1 did not reach a maximal level in the early stage of acid production but continued to increase well into the period of more rapid acid production, indicating the requirement of oxygen for peroxide production. The adverse effect of aeration on the acid production by *S. lactis* AC1 was apparently due to more than the formation of peroxide since the prevention of peroxide accumulation by catalase did not result in greater stimulation than was observed with the static cultures. Molecular oxygen can oxidize enzyme sulfhydryl groups, resulting in reduced enzyme activity (9, 15). The increased amount of oxygen in the aerated system may have been sufficient to oxidize essential sulfhydryl group-containing enzymes required for optimal acid production. The intracellular proteinase of the lactic streptococci, which is necessary for optimal culture activity, is susceptible to inactivation by oxidation (1).

According to George (6), ferrous ions break down hydrogen peroxide in a reaction similar to catalase. Ferrous sulfate prevented the accumulation of peroxide by *S. lactis* AC1 and had a stimulatory effect on the culture. The stimulatory response obtained by adding ferrous sulfate to the culture was greater than that obtained by adding catalase, indicating that more was involved in the stimulation by ferrous sulfate than the destruction of metabolically produced peroxide. Cowman and Speck (1) reported that ferrous ions increased the proteolytic activity of *S. lactis*, which resulted in faster growth and acid production.

The stimulatory response of lactic streptococci to exogenously added catalase is apparently due to the destruction of metabolically produced peroxide. The production of autoinhibitory levels of peroxide offers an explanation for slow rates of acid production exhibited by some lactic streptococci.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**