OXYTETRACYCLINE-BINDING PROPERTIES
OF STREPTOCOCCUS LACTIS

Previous studies in our department have indicated differences between the cell walls of oxytetracycline-resistant and oxytetracycline-susceptible strains of *Streptococcus lactis* in respect to permeability (4) and to amino acid content (2). In a recent paper, Izaki and Arima (1) report that *Escherichia coli* cells will accumulate oxytetracycline when exposed to high concentrations of antibiotic (100-400 μg/ml). The present study was conducted to determine the cell-site of antibiotic accumulation using radioactive oxytetracycline.

**EXPERIMENTAL PROCEDURE**

*S. lactis* oxytetracycline-resistant and -susceptible strains were those described by Shahani and Harper (4).

The cells used in the tracer studies were prepared from a 16-18 hr growth in hydrolyzed milk protein broth described by Sobol (5). The cells were sedimented from the broth by centrifugation. The broth discarded and the packed cells were washed with sterile distilled water to remove any adhering nutrients. The cell suspension was centrifuged and the wash water discarded. The washing and centrifugation steps were repeated twice. After the third washing, the packed cells were suspended in 0.85% sterile saline solution to approximately the same viable cell concentration.

Randomly labeled C\(^14\)-oxytetracycline was added to the cell suspension to produce a final antibiotic concentration of 1 μg/ml. The radioactivity of the cell-saline-oxytetracycline mixture was determined immediately, after which the mixture at 30 C was either (a) centrifuged following 3 min holding or (b) held for 30 min prior to centrifugation. Centrifugation was at 2,900 × g in a Lourdes Model AB Angle Centrifuge. Following centrifugation, the radioactivity of the supernatant was determined, the supernatant discarded, and the packed cells resuspended in fresh saline. The sequence was repeated twice, with the supernatant being subjected to radioactivity measurements in each case. After the second washing, the cells resuspended in fresh saline were ruptured by sonic vibration to an efficiency of over 90% destruction as determined by viable cell count. Sonic disintegration of cells was accomplished by a Raytheon 200 watt, 10 Kc Magnetostriction Oscillator, with an exposure time of 15 min. The solution was then centrifuged for 5 min at 2,900 × g and in some cases at 14,000 × g. Radioactivity of the sediment was measured. A Nuclear (Chicago) Model 181A Scaler with a windowless gas flow detector was used to measure radioactivity, reported as counts per minute (cpm) per 0.1 ml supernatant or per 10 mg sediment.

**RESULTS AND DISCUSSION**

Oxytetracycline-binding was investigated for antibiotic-resistant and -susceptible *S. lactis* cells exposed to radioactive oxytetracycline for 3 min or for 30 min. The cell-site of oxytetracycline accumulation was determined by measuring the amount of radioactivity in the sonically ruptured cell fractions separated by centrifugation at either 2,900 or 14,900 × g.

The results (Table 1) reveal that the radioactivity of the initial contact mixture (consisting of bacterial cells in a saline solution containing C\(^14\)-oxytetracycline) did not vary by more than 10% under all experimental conditions and that from 22-44% of the oxytetracycline was adsorbed and could not be removed by washing. By the second washing, the radioactivity of the supernatant had decreased to 4-10 cpm above background.

The radioactivity of the cytoplasm of the sonically ruptured cells contained slightly more oxytetracycline (10 cpm) when the cells were exposed for 3 min to the antibiotic, as compared to the cytoplasm of the sonically ruptured cells exposed for 30 min to the antibiotic.

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxytetracycline-resistant cells</th>
<th>Oxytetracycline-susceptible cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure time</td>
<td>3 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Cells in saline with oxytetracycline</td>
<td>291</td>
<td>283</td>
</tr>
<tr>
<td>Supernatants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadsorbed</td>
<td>164</td>
<td>222</td>
</tr>
<tr>
<td>First washing</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Second washing</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Supernatant after cell rupture (cytoplasm)</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>Sediment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 2,900 × g</td>
<td>133</td>
<td>107</td>
</tr>
<tr>
<td>At 14,000 × g</td>
<td>308</td>
<td>383</td>
</tr>
</tbody>
</table>

\(^*\)Averages of three separate trials.

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1. Article 17:63. This investigation was supported by Public Health Services Grant No. EF 00101 from the Division of Environmental Engineering and Food Protection.
pared with a 30-min exposure period. Under all conditions, the radioactivity of the cytoplasmic material ranged from 21-31 cpm, indicating that oxytetracycline fails to penetrate the osmotic barrier of either strain and that the 30-min antibiotic exposure period decreased slightly the cytoplasmic concentration of oxytetracycline.

Counts on the accumulated sediment after centrifugation at 2,900 × g of sonically ruptured cells showed that the amount of oxytetracycline present in this fraction did not vary appreciably between the 3- and 30-min exposure periods within individual strains. However, variations were noted in the amount of antibiotic bound by resistant and susceptible cells. From 1.5 to 1.8 times as much oxytetracycline was taken up by the resistant cells as by the susceptible cells.

In most cases, the use of 14,000 × g force was found to increase the amount of radioactivity present in the cell sediment. In every instance, the resistant cells exhibited more radioactivity than their susceptible-cell counterparts. However, differences between the two strains in the amounts of activity present in the sediment decreased markedly after the cells had been exposed to radioactive oxytetracycline for 30 min. For the cells exposed for 3 min to radioactive oxytetracycline, the radioactivity ratio of resistant to susceptible-cell sediment was 5.3:1. When the cells were held for 30 min in the radioactive medium, the ratio of resistant to susceptible cells sediment was only 1.4:1.

Actual activity values for the resistant cell sediment separated at 14,000 × g force were increased from 308 cpm for the 3-min exposure to 383 cpm for 30-min exposure, an increase of 26% cpm; whereas, the susceptible-cell sediment under similar conditions increased 217 cpm, from 58 to 275 cpm. Thus, it would appear that a suitable incubation period is required for binding of antibiotic by susceptible cells; whereas, such a time requirement for binding is not evident for the resistant strain.

**SUMMARY**

Antibiotic-binding differences between an oxytetracycline-resistant and -susceptible strain of *S. lactis* were studied by use of radioactive tracer techniques. C14-oxytetracycline was bound tenaciously by both the antibiotic-resistant and antibiotic-susceptible strains. The resistant cells bound a greater quantity of the antibiotic than the susceptible cells, with this binding being particularly marked when oxytetracycline was permitted 3-min contact with the cells. Radioactive oxytetracycline accumulated in the cell fraction, which was sedimented by centrifugation of sonically ruptured cells at 14,000 × g. Oxytetracycline concentration in the cytoplasm of either the resistant or susceptible cells was negligible.

E. M. MIKOLAJCIK
W. J. HARPER
AND
I. A. GOULD
Department of Dairy Technology
The Ohio State University, Columbus

**REFERENCES**


(2) **MIKOLAJCIK, E. M. 1965. Physiology of Oxytetracycline Resistant and Sensitive Streptococcus lactis. Ph.D. thesis, The Ohio State University, Columbus.**


**DESTRUCTION OF THE PROPERTIES OF SOME ANTIBIOTICS BY HYDROGEN PEROXIDE**

Hydrogen peroxide destroys the antibiotic properties of penicillin (2) and the lactenins (1). This study was carried out to determine the effect of *H₂O₂* on other common antibiotics. This information may aid in distinguishing between the various antibiotics present in milk or other fluids. Further, *H₂O₂* treatment of milk containing antibiotics may permit the use of such milk for cheese-making.

**METHODS**

Skimmilk containing either penicillin, aureomycin, streptomycin, or terramycin was treated with *H₂O₂* under the conditions given with each experiment. After the desired period of time the residual *H₂O₂* was destroyed by added catalase. The concentration of residual antibiotic was determined by the Reverse Phase Assay (3), using *B. subtilis* as test organism and 0.63-cm discs.

**RESULTS**

Of the four antibiotics tested, penicillin proved to be the most susceptible and streptomycin least, Table 1. In this experiment an incubation temperature of 45°C was used, since