# THE LACTIC ACID FERMENTATION OF STREPTOCOCCI

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Although all true streptococci are assumed to be homofermentative organisms which yield predominantly lactic acid in the fermentation of carbohydrates, exact data are available on only a few species. More than thirty years ago Zuzuki, Hastings and Hart (1910) showed that *Streptococcus lactis* produces from 90 to 99 per cent lactic acid from the sugar fermented. In the recent important works of Hewitt (1932) and Friedemann (1938, 1939a, b, c) on hemolytic streptococci (probably mostly group A) and a few non-hemolytic types, Hewitt reported from 68 to 85 per cent lactic acid, whereas Friedemann obtained results ranging from 75 to approximately 100 per cent.

That all streptococci may not be strictly homofermentative under all conditions is indicated by several reports. Although Hewitt found little difference between aerobic and anaerobic cultures by the methods used by him, Friedemann obtained a reduction in yield of lactic acid from 85 to 47 per cent in very thoroughly aerated cultures. In a preliminary note, Davis and Rogers (1939) have reported that with the cell suspension technique the enterococci under aerobic conditions give a yield of only about 50 per cent of lactic acid. Bearing in a general way on the problem is the finding of Niven, Smiley and Sherman (1941) that *Streptococcus salivarius* when grown in *sucrose* media may utilize as much as 40 per cent of the sugar in the synthesis of a polysaccharide.

The present study of lactic acid production by streptococci was undertaken in order to obtain data on all of the better known species. Thus it was hoped to learn whether or not all types of streptococci are strictly homofermentative when glucose is utilized under anaerobic conditions, and, further, if all species are equally efficient in the production of lactic acid.

## METHODS

The cultures were transferred three times before being used, the last transfer being made 12 hours before inoculation into the flasks from which the cell crops were to be obtained. The medium used for transferring the cultures and for growing the cell crops contained, per liter:

| | | |
|---|---|---|
| Glucose | 1 | gram |
| Tryptose (Difco) | 10 | grams |
| Meat infusion | 500 | ml. |
| Tomato juice | 25 | ml. |
| Water to volume; pH, | 7.0 | |

A flask containing 250 ml. of this medium was used to grow the cells for one fermentation. This was inoculated with 5 ml. of the 12-hour culture and incubated at 37°C. for 12 hours. The pH of the medium after growth was completed varied between 5.0 and 5.5. This did not affect adversely the activity of the cells of the majority of organisms used but reduced the activity of some strains of the pyogenic and viridans groups. It was then found that shortening the growth time of these species which showed reduced activity after 12 hours to between 10 and 11 hours gave cells of high activity.

At the end of the growth period the cells were removed by centrifugation, resuspended in 10 ml. of an 0.85 per cent sodium chloride solution, and spun down again. The cells were then suspended in 5 ml. of saline solution, and placed in a 100 ml. volumetric flask containing exactly 50 ml. of the sugar solution to be fermented. This substrate solution contained:

| | |
|---|---|
| Glucose (anhydrous) | 0.5 per cent |
| $K_2HPO_4$ (anhydrous) | 1.0 per cent |
| $KH_2PO_4$ (anhydrous) | 0.5 per cent |

The pH of the substrate solution was 7.0. The constituents were carefully weighed on a torsion balance and the sugar concentration varied only within the range of 246 to 251 mgm. per 50 ml.

After addition of the cells to the substrate, the flasks were incubated at 37°C. for 12 hours. It was found that an active suspension will ferment substantially all of the sugar in four to

five hours; however, the time was extended to 12 hours to enable the slow cultures to complete fermentation. At the end of this period, 5 ml. of 20 per cent sulfuric acid were added to the flask, which was then made up to 100 ml. and stoppered until analyzed.

In making determinations without anaerobic precautions the flasks containing the cells and glucose solution were not protected from air, but they were under essentially anaerobic conditions. In the anaerobic fermentations the flasks were placed in a vacuum desiccator and the air removed with an oil pump.

Lactic acid determinations were made using the modifications developed by Troy and Sharp (1935) in which the bacterial cells, proteins, glucose and other interfering substances are precipitated with copper hydroxide (copper sulfate-calcium hydroxide) at 45°C. In this method the lactic acid is oxidized to acetaldehyde, the aldehyde trapped in a bisulfite solution, and titrated with iodine.

Analyses for glucose were made by the micro method of Shaffer and Somogyi (1933). This is in principle an iodometric procedure following copper reduction.

## RESULTS

In all, 151 cultures representing the better known species and varieties of streptococci have been studied. A total of 286 tests were made, each test being a separate experiment run with independently grown and prepared cells. At the outset, fermentations were conducted under strictly anaerobic conditions on the various species, 81 of these tests being made. As no detectable difference in yield was obtained between these tests and those run without anaerobic precautions, the fermentations under strictly anaerobic conditions were discontinued. In the data reported (table 1) the anaerobic results are included in the averages.

It appears clear that all of the well known species of the true streptococci are homofermentative, in the usual sense of this term, when acting on glucose under substantially anaerobic conditions. It is hoped that work now under way in this laboratory will indicate to what extent the fermentation may be modified under certain other conditions.

The slightly lower average efficiency of the pyogenic streptococci is interesting and probably of significance. Although there was of course some overlapping of individual determinations,

TABLE 1

*Lactic acid production from glucose by streptococci*

(Per cent lactic acid per unit of glucose fermented)

| GROUP, SPECIES OR VARIETY | TOTAL CULTURES | TOTAL TESTS | AVERAGE YIELD |
|---|---|---|---|
| | | | *per cent* |
| Pyogenic streptococci | | | |
| *Group A (S. pyogenes and varieties)* | 6 | 21 | 87.6 |
| *Group B (S. mastitidis and varieties)* | 12 | 21 | 89.7 |
| *Group C ("animal")* | 6 | 14 | 85.8 |
| *Group C ("human")* | 6 | 15 | 87.5 |
| *Group E* | 2 | 7 | 87.8 |
| *Group F* | 2 | 5 | 81.8 |
| *Group G ("minute")* | 4 | 7 | 87.1 |
| *Group G ("non-minute")* | 8 | 20 | 87.7 |
| *Group H* | 2 | 5 | 87.6 |
| Viridans streptococci | | | |
| *Streptococcus salivarius* | 12 | 18 | 93.6 |
| *Streptococcus equinus* | 12 | 25 | 92.3 |
| *Streptococcus bovis* | 12 | 18 | 91.7 |
| *"Bargen streptococcus"* | 2 | 4 | 91.9 |
| *Streptococcus thermophilus* | 2 | 2 | 90.2 |
| Lactic streptococci | | | |
| *Streptococcus lactis* | 12 | 18 | 96.6 |
| *Streptococcus cremoris* | 12 | 18 | 93.7 |
| Enterococci (group D) | | | |
| *Streptococcus fecalis* | 12 | 18 | 96.0 |
| *Streptococcus liquefaciens* | 5 | 10 | 91.2 |
| *Streptococcus durans* | 12 | 18 | 92.2 |
| *Streptococcus zymogenes* | 12 | 18 | 95.8 |
| *Streptococcus uberis* | 2 | 4 | 90.8 |

the difference appears to be real. It is true that vigorous fermentations with practically complete consumption of the sugar are more apt to give higher percentage yields of lactic acid; and the pyogenic streptococci usually fermented less vigorously with

incomplete utilization of the glucose. For example, the two group F cultures gave very feeble fermentations and low percentage yields of lactic acid. As a complete explanation, however, this thesis does not appear to bear analysis. From 21 tests with *Streptococcus pyogenes* (group A) minimum, maximum and average yields of 81.7, 92.3 and 87.6 per cent were obtained. In seven of the experiments with this species the fermentations were very active with substantially complete utilization of the glucose, but the average yield of lactic acid in these seven tests was only 88.7 per cent. In contrast, *Streptococcus lactis* on 18 tests gave minimum, maximum and average yields of 93.8, 99.6 and 96.6 per cent. One culture of *Streptococcus lactis* fermented weakly with very low utilization of the glucose, but the percentage yield of lactic acid was 94.6. Since the publication of a preliminary abstract of this work (Smith and Sherman, 1941) many additional experiments have been run with the pyogenic streptococci and it is now felt that the lower yields given by them are of significance, though admittedly the true significance may not be so simple as that implied by these results.

TABLE 2

*Lactic acid yields from cultures and cell suspensions*

(Per cent lactic acid per unit of glucose fermented)

| ORGANISM | GROWING CULTURE | RESTING CELLS |
|---|---|---|
| | *per cent* | *per cent* |
| *Streptococcus lactis* (L21) | 97.1 | 98.8 |
| *Streptococcus fecalis* (718) | 96.3 | 94.7 |
| *Streptococcus zymogenes* (5C1) | 92.9 | 93.1 |
| *Streptococcus salivarius* (S30A) | 93.4 | 93.5 |

Inasmuch as previous workers have used growing cultures in investigations of this type a few tests were made in order to find how closely such results check with the so-called resting cell technique. The cultures were incubated 48 hours at 37°C. in a medium containing 0.25 per cent glucose and 0.5 per cent tryptose; with the results given in table 2.

It is apparent that satisfactory results may be obtained with

ordinary culture methods; and that the method used for lactic acid determinations is adequate to take care of the interfering substances in a relatively simple culture medium. If these few tests may be relied upon, it would appear that the proportion of sugar used for cell synthesis is exceedingly small.

## SUMMARY

Washed cells were suspended in a phosphate-buffered glucose solution; a total of 286 tests being made with 151 cultures representing the better known groups and varieties of streptococci. Similar results were obtained under strictly anaerobic conditions and without anaerobic precautions but the latter fermentations were essentially anaerobic. The average per cent lactic acid from glucose fermented for each variety follows.

Pyogenic streptococci: Group A, 87.6; group B, 89.7; group C, "animal," 85.8; group C, "human," 87.5; group E, 87.8; group F, 81.8; group G, "minute," 87.1; group G, "non-minute," 87.7; group H, 87.6.

Viridans streptococci: *Streptococcus salivarius*, 93.6; *Streptococcus bovis*, 91.7; "Bargen streptococcus," 91.9; *Streptococcus equinus*, 92.3; *Streptococcus thermophilus*, 90.2.

Lactic streptococci: *Streptococcus lactis*, 96.6; *Streptococcus cremoris*, 93.7.

Enterococci (group D): *Streptococcus fecalis*, 96.0; *Streptococcus liquefaciens*, 91.2; *Streptococcus zymogenes*, 95.8; *Streptococcus durans*, 92.2; *Streptococcus uberis*, 90.8.

The slightly lower average efficiency of the pyogenic streptococci is of possible significance.

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