THE FERMENTATION OF DISACCHARIDES BY STREPTOCOCCUS THERMOPHILUS

J. M. SHERMAN AND PAULINE STARK

Laboratory of Bacteriology, College of Agriculture, Cornell University, Ithaca, New York

Received for publication February 2, 1938

Orla-Jensen (1919) was the first to differentiate and describe *Streptococcus thermophilus* as a distinct streptococcal species. Among the unique characteristics of this organism which were pointed out by Orla-Jensen are its general inability to ferment maltose, and its ability to ferment sucrose and lactose with especial vigor, the latter disaccharides being even more favorable to the growth of this organism than are the simple monosaccharides. Orla-Jensen also noted the very interesting fact that *Streptococcus thermophilus* does not ferment mannose, or ferments it very feebly.

Wright (1936) has also noted that some strains of *Streptococcus thermophilus* ferment lactose and sucrose more rapidly than they do the constituent monosaccharides, and on the basis of these results he suggests that *Streptococcus thermophilus* ferments the disaccharides without preliminary hydrolysis. In times past it was a common belief that the lactic-acid bacteria caused a direct fermentation of the disaccharides, since the presence of monosaccharides cannot usually be demonstrated in fermenting cultures by chemical means; even then, however, most investigators held to the theory that the disaccharides were hydrolyzed to the constituent monosaccharides as the first step in their fermentation.

Some years ago one of us became interested in this problem in connection with *Streptococcus lactis*. It was thought that although the preliminary hydrolysis of the disaccharides was not easily proved by chemical means, an indication that such hy-

drolysis actually occurs might be demonstrated by the use of bacteria. It was found that if a culture of *Streptococcus lactis* (sucrose +) and a culture of *Bacterium coli* (sucrose −) are grown together in sucrose broth, gas is formed, though neither of these organisms alone can produce gas from sucrose. Also, when *Streptococcus lactis* (lactose +) and *Proteus* (lactose −) are grown together in lactose broth, gas is likewise produced. At that time it did not appear that this fact had been demonstrated with *Streptococcus lactis*. However, our results were not published, because a survey of the literature showed that such phenomena had been encountered in connection with "false presumptive tests" for *Bacterium coli* in water, in which cases the gas produced from lactose appeared to result from the cooperative action of such organisms as *Streptococcus fecalis*, which can ferment lactose without gas production, and other bacteria capable of producing gas from glucose though not attacking lactose. These facts naturally suggested themselves in connection with Wright's idea concerning the direct fermentation of disaccharides by *Streptococcus thermophilus*.

That *Streptococcus thermophilus* appears to utilize sucrose and lactose more readily than the monosaccharides is easily demonstrated by the rate of acid production in media containing these sugars. Table 1 shows the relative rates of fall in pH with two cultures of *Streptococcus thermophilus* in broths containing 1 per cent of the respective sugars together with 0.3 per cent each of beef extract, peptone and tryptone. Galactose, not included in the table, was fermented more slowly than glucose, as Wright has previously shown.

Streptococcus thermophilus and *Bacterium coli* were grown separately and together in sucrose broth. Since the strain of *Bacterium coli* used could not ferment sucrose, neither of these organisms alone is able to produce gas from this sugar whereas gas production resulted when the two organisms were grown together. The results obtained are shown in table 2.

Experiments were also conducted with *Streptococcus thermophilus* and *Proteus* (lactose −) in lactose broth. The findings (table 3) are in agreement with those obtained with sucrose.

Tests similar to those reported in tables 2 and 3 were conducted with two strains of *Streptococcus thermophilus* and the experiments were repeated a number of times in media of different composition.

TABLE 1

The rate of fermentation (pH) of hexoses and disaccharides by Streptococcus thermophilus

CULTURE	HOURS	GLUCOSE	FRUCTOSE	SUCROSE	LACTOSE
2	0	6.6	6.6	6.6	6.6
	24	6.4	6.2	5.2	5.3
	66	6.0	4.4	4.7	4.3
	168	5.0	4.4	4.5	4.2
6	0	6.6	6.6	6.6	6.6
	24	6.5	6.2	5.3	5.4
	66	6.0	4.4	4.7	4.3
	168	5.0	4.4	4.5	4.2

TABLE 2

Fermentation of sucrose by Streptococcus thermophilus and Bacterium coli

INOCULUM	ACID	GAS
S. thermophilus	+	−
Bact. coli	−	−
S. thermophilus + *Bact. coli*	+	+

TABLE 3

Fermentation of lactose by Streptococcus thermophilus and Proteus

INOCULUM	ACID	GAS
S. thermophilus	+	−
Proteus	−	−
S. thermophilus + *Proteus*	+	+

The formation of gas was revealed by the use of test tubes of broth with vaseline seals, but in some tests it was also demonstrated with the use of inverted Durham tubes.

To repeat these results, a little attention should be paid to the

composition of the medium. In a lightly buffered medium, if *Streptococcus thermophilus* gets a rapid start the associated organisms may be inhibited before visible evidence of gas is obtained. On account of the extreme sensitiveness of *Streptococcus thermophilus* to salt (Sherman, 1937), it may not do well in media heavily buffered with phosphates. We have found a suitable medium to be one containing 1 per cent of the test sugar with 0.3 per cent each of beef extract, peptone, tryptone, and dibasic potassium phosphate, though several other media have been used successfully.

Although the name *Streptococcus thermophilus* has been loosely used by some workers for various heat-tolerant streptococci, this organism has a very peculiar combination of characteristics which widely separates it from the other clearly defined streptococcal types. In our own work covering a considerable experience in addition to our published data (Sherman and Stark, 1931), the various strains studied have made up a remarkably homogeneous group, though somewhat greater variation has been noted by others who have undoubtedly dealt with the true *Streptococcus thermophilus*.

With reference to the fermentation of disaccharides by *Streptococcus thermophilus*, these simple experiments should not be accepted as offering definite proof; they do, however, indicate that this organism probably hydrolyzes the disaccharides to their constituent monosaccharides as the initial step in the fermentation; they do, also, put the burden of proof on those who hold the opposite view.

SUMMARY

It is shown that if *Streptococcus thermophilus* (sucrose +) and *Bacterium coli* (sucrose −) are grown together in sucrose broth, gas is produced, whereas neither of these organisms alone can produce gas from sucrose. Likewise, gas is produced in lactose broth when *Streptococcus thermophilus* (lactose +) and *Proteus* (lactose −) are grown together.

These results suggest, in opposition to one view which has been held in the past, that *Streptococcus thermophilus* probably hy-

drolyzes the disaccharides to their constituent monosaccharides in the fermentation of such sugars.

REFERENCES

ORLA-JENSEN, S. 1919 The lactic acid bacteria. Copenhagen.

SHERMAN, J. M. 1937 The streptococci. Bact. Reviews, **1**, 1–97.

SHERMAN, J. M., AND STARK, PAULINE 1931 Streptococci which grow at high temperatures. Jour. Bact., **22**, 275–285.

WRIGHT, H. D. 1936 Direct fermentation of disaccharides and variation in sugar utilization by *Streptococcus thermophilus*. Jour. Path. Bact., **43**, 487–501.