FERMENTATION OF SUGAR ACIDS BY BACTERIA

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Bacterial fermentation of the sugar acids has received little attention in comparison with the wide-spread use of the common aldoses and sugar alcohols. These acids, and especially their sodium salts, are well suited to bacteriological techniques because of their solubility and heat stability, yet their usefulness for investigating the relationship of chemical structure to biological utilization has only recently been recognized. Their application to systematic differentiation of bacteria has also been neglected.

Kendall, Bly and Haner (1923) concluded from a study of three monocarboxylic acids and three dicarboxylic acids that these compounds possessed little value for the differentiation of bacterial species.

Koser and Saunders (1933) included one monocarboxylic acid with many other derivatives, and Sternfeld and Saunders (1937, 1938) employed seven monocarboxylic acids and one dicarboxylic acid in their studies on the utilization of sugars and their derivatives by bacteria. General conclusions concerning the relationship of configuration to fermentation were drawn, and the potential diagnostic value of certain compounds, especially mucic acid, was pointed out.

The present study involved the use of two dicarboxylic acids, mucic and d-saccharic acids, and ten monocarboxylic acids, d-gluconic, d-mannonic, d-galactonic, d-talonic, l-gluconic, l-mannonic, l-rhamnonic, 5-keto-d-gluconic, d-arabonic and l-arabonic acids. The fermentation reactions of the latter seven had not previously been studied. Related aldoses and sugar

alcohols were also included for comparison with the acids. It was hoped to demonstrate additional facts concerning the relation of structure to fermentation of carbohydrates, and to find possible differential fermentations for distinguishing between bacterial species.

EXPERIMENTAL

Twenty-four species of bacteria representing eleven genera were selected as test organisms from the stock culture collection maintained in the Department of Bacteriology.

The physical properties of the carbohydrates[1] used agreed with accepted values for the melting points and specific rotations found in the literature.

The medium employed for all tests was prepared as follows:

Bacto peptone	1%
Bacto beef extract	1%
Cysteine hydrochloride	0.05%
Test carbohydrate	0.5%
Distilled water	q.s.

Warm gently to dissolve ingredients, adjust to pH 7.2–7.4, add 1 ml. per liter of 1.6 per cent alcoholic solution of bromcresol purple, dispense in approximately 6 ml. amounts to small Durham tubes, and sterilize in the autoclave at 15 pounds pressure for 15 minutes.

The crystalline aldoses and alcohols were added directly to the medium, while the acids were first converted to the sodium salts. This was done by warming the acid or its lactone with slightly more than an equivalent amount of sodium hydroxide or sodium bicarbonate solution.

Heat sterilization was considered satisfactory since sugar acid salts are highly resistant to decomposition by heat, even in moderately acid or alkaline solutions. This assumption is also supported by the findings of Sternfeld and Saunders (1937), who noted no difference in fermentation between autoclaved and filtered media containing these substances.

Duplicate tubes of each medium were inoculated with one

[1] Dr. F. W. Upson of the Department of Chemistry, University of Nebraska, kindly supplied several of the compounds used in the study.

loopful of twenty-four hour old broth cultures, and were incubated at 37°C. All cultures of *Lactobacillus* and *Propionibacterium* species were incubated anaerobically in the presence of 10 per cent carbon dioxide according to the method of Weiss and Spaulding (1937).

Uninoculated tubes of each carbohydrate medium and inoculated tubes of carbohydrate-free medium served as controls.

Observations were made daily for the first week, at three-day intervals during the second week, and a final reading was taken after three weeks. Growth was determined by the appearance of turbidity. The criteria of fermentation were the occurrence of gas in the inverted vials and change of the bromcresol purple indicator to the yellow color characteristic of acid reactions.

The results recorded in table 1 are in general agreement with those of Sternfeld and Saunders (1937). However, these authors did report that l-rhamnose and d-galactose were not utilized by *Proteus vulgaris* and that acid only was produced by this organism from d-glucose and d-mannose. In contrast, our strain of *Proteus vulgaris* formed both acid and gas from each of these aldoses. They also found that *Shigella sonnei* fermented l-rhamnose, that *Staphylococcus aureus* fermented d-gluconic acid but not d-mannose and d-galactose, and that *Staphylococcus albus* fermented sorbitol, dulcitol, and mucic acid, while opposite results were observed in this study. These and other discrepancies are probably due to inherent differences between individual strains of the species used in the two studies.

The findings of Kendall, Bly and Haner (1923) are not in good agreement either with our observations or with those of Sternfeld and Saunders (1937), but as the latter authors have pointed out, the improper selection of the pH indicator may have given unreliable results in the former work.

Useful tests for distinguishing between bacterial species on the basis of fermentation were suggested in certain cases. Further study with more strains of each species involved would be necessary, however, to establish their validity. Production of acid from sorbitol and not from d-galactonic acid might serve to differentiate *Eberthella typhosa* from *Shigella dysenteriae* and

Shigella sonnei which produced acid from d-galactonic acid but not from sorbitol. Fermentation of d-mannonic acid, as well as mannitol and sorbitol, by *Streptococcus lactis* and *Streptococcus liquefaciens* could, perhaps, be used to aid in distinguishing these

TABLE 1

Acid and gas production from carbohydrates and their derivatives by twenty-four species of bacteria

SPECIES	d-GLUCOSE	d-SORBITOL	d-GLUCONIC ACID	d-SACCHARIC ACID	d-MANNOSE	d-MANNITOL	d-MANNONIC ACID	d-GALACTOSE	DULCITOL	d-GALACTONIC ACID	MUCIC ACID	l-RHAMNOSE	l-ARABONIC ACID
Aerobacter aerogenes	AG	AG	AG	AG	AG	AG	AG	AG	0	0	AG	AG	0
Aerobacter cloacae	AG	AG	AG	A	AG	AG	AG	AG	0	AG	AG	AG	0
Escherichia coli	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	0
Proteus vulgaris	AG	0	AG	0	AG	0	AG	AG	0	0	0	AG	0
Salmonella paratyphi	AG	A	AG	0	AG	AG	A	AG	AG	0	0	AG	0
Salmonella schottmulleri	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	0	AG	A
Salmonella pullorum	AG	A	AG	AG	AG	AG	0	AG	0	AG	0	AG	0
Eberthella typhosa	A	A	A	0	A	A	0	A	A	0	0	0	0
Shigella dysenteriae	A	0	A	0	A	0	0	A	0	A	0	0	0
Shigella sonnei	A	0	A	0	A	A	0	A	0	A	0	0	0
Staphylococcus aureus	A	0	0	0	A	A	0	A	0	0	0	0	0
Staphylococcus albus	A	0	A	0	A	A	0	A	0	0	0	0	0
Streptococcus lactis	A	A	0	0	A	A	A	A	0	0	0	0	0
Streptococcus liquefaciens	A	A	0	0	A	A	A	A	0	0	0	0	0
Streptococcus agalactiae	A	0	0	0	A	0	0	A	0	0	0	0	0
Lactobacillus delbrueckii	A	A	0	0	A	A	0	A	A	A	0	0	0
*Lactobacillus acidophilus**	A	0	0	0	A	0	0	A	0	0	0	0	0
*Lactobacillus bifidus**	A	0	0	0	A	0	0	A	0	0	0	0	0
Propionibacterium technicum	A	0	A	0	A	A	A	AG	0	0	0	0	0
Propionibacterium zeae	A	A	A	0	A	A	A	A	0	0	0	AG	0
Propionibacterium pentosaceum	A	A	AG	0	A	AG	A	A	0	0	0	AG	0
Corynebacterium xerose	A	0	0	0	A	0	0	0	0	0	0	0	0
Corynebacterium pseudodiphthericum	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynebacterium diphtheriae	A	0	0	0	0	0	0	0	0	0	0	0	0

A = acid production. G = gas production. 0 = no acid or gas production.

* Growth occurred only when carbohydrate was fermented.

No fermentation by any organism was observed for d-talonic, l-gluconic, l-mannonic, l-rhamnonic, 5 keto-d-gluconic and d-arabonic acids.

organisms from *Streptococcus agalactiae*. It was also thought that acid production from d-gluconic and d-mannonic acids might prove to differentiate the genus *Propionibacterium* from *Lactobacillus* but more extensive study showed this to fail for certain species within each genus.

The general conclusion of Sternfeld and Saunders (1937) that

any change from the structure of the aldose results in decreased frequency of fermentation for the derivative is in agreement with our observations. Thus d-gluconic, d-mannonic, d-galactonic and l-rhamnonic acids as well as d-sorbitol, d-mannitol and dulcitol were fermented by fewer organisms than the corresponding aldoses. Similarly, the dicarboxylic mucic and d-saccharic acids were fermented less often than the corresponding monocarboxylic d-galactonic and d-gluconic acids. It should be noted that, although the acids were utilized by fewer bacterial species than the aldoses, no prediction concerning the degree of availability of the acids could be made from the fermentation of the corresponding aldoses. Thus d-mannose, d-galactose and l-rhamnose were fermented by twenty-two, twenty-one and nine species respectively, while the corresponding acids were fermented by eleven, seven and none. Also, Sternfeld and Saunders (1937) reported both d- and l-arabinose to be fermented by numerous organisms, while we found l-arabonic acid to be fermented by only one species and d-arabonic acid by none.

The cases cited in the literature, where enantiomorphic aldoses were studied, have indicated fermentation of both forms. Thus, the two arabinoses and the two riboses were reported by Sternfeld and Saunders (1937) to be attacked by many species, the naturally occurring forms being fermented by a few more organisms than their isomers. Hence, the complete lack of fermentation of l-gluconic and l-mannonic acids was somewhat unexpected, since the d-form of both was fermented by many species.

Definite correlation of the asymmetric structure of the sugar acids with their utilization by bacteria was not obtained. Comparison of the fermentations of d-gluconic and d-mannonic acids shows several differences which may be attributed to the change in configuration at the second carbon atom. Still greater differences due to the configuration on the second carbon atom were observed with the epimers, d-galactonic and d-talonic acids. The former was fermented by seven species and the latter by none. No regularity concerning the utilization of acids with either cis or trans configuration of the hydroxyls on the second and third

carbons was noted. Furthermore, comparisons of d-gluconic acid with d-galactonic acid and of d-mannonic acid with d-talonic acid showed that changes in configuration on the fourth carbon atom were significant in determining fermentation. It would seem, therefore, that fermentation of sugar acids by bacteria is conditioned by the total structure of the molecule rather than any key structures such as Armstrong and Armstrong (1934) have described for emulsin.

SUMMARY

1. The fermentative action of 24 species of bacteria on two dicarboxylic and ten monocarboxylic sugar acids, including seven compounds not previously reported, is compared with the utilization of related aldoses and sugar alcohols.

2. The conclusion of others that any change from the structure of the aldose lessens the frequency of utilization is substantiated in several further instances.

3. The usefulness of sugar acids for distinguishing between certain bacterial species is indicated as being worthy of future study.

4. The relation of asymmetric structure to fermentation of sugar acids is not entirely proved, but indications are that total structure of the molecule conditions availability.

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