THE UTILIZATION OF CERTAIN SUGARS AND THEIR DERIVATIVES BY BACTERIA

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Although the commoner sugars and alcohols have found an everyday use in bacteriological work for the differentiation and characterization of species, it is only rarely that sugar fermentation has been studied from the standpoint of correlating sugar structure with utilization.

In the present investigation we have employed a number of "substituted" sugars and have compared the ability of various bacteria to make use of these modified sugar molecules with the utilization of the commoner sugars from which they were derived. The sugars available for this study comprised a number of methyl derivatives of the commoner hexoses and pentoses, an amino derivative, a sugar acid, a sulphur-containing sugar, several acetyl sugars and two heptoses. These were subjected to tests of fermentation by a number of the commoner saprophytic and pathogenic bacteria.

METHODS

Utilization of the sugars was determined by the conventional method of detecting acid and gas production by the organisms when grown in a suitable medium containing a small amount of the test substance. There is of course the possibility that certain organisms might make use of the sugars without giving rise to detectable acid end-products and that this would lead to erroneous conclusions. It is believed, however, that the usual method of detecting fermentation is substantially accurate for the microorganisms used in this study. Moreover, the alternative method

of quantitative estimation of each test substance was out of the question since accurate methods are not available for all of the rare sugars or sugar derivatives in a substratum such as nutrient broth.

In the preparation of the rare sugars used for this investigation the usual methods were followed and the melting points, after repeated crystallizations, were checked with those reported in the chemical literature.[1]

Liquid media were employed for most of the fermentation tests, though a semi-solid agar was also used on some occasions. For the basic medium, either meat-extract-peptone broth or veal-infusion-peptone broth was used, depending upon the growth requirements of the various organisms to be tested. In every case brom-thymol-blue was added and the medium adjusted to pH 7.0. The resulting indicator broth was tubed in definite amounts and sterilized in the autoclave. In a few instances, especially when pneumococci were employed, 10 per cent by volume of sterile beef serum was added to the infusion broth after sterilization.

Solutions of the sugars and related compounds were prepared separately in distilled water and sterilized by filtration through Seitz filters. They were then added to the indicator medium to give concentrations of 0.5 to 1.0 per cent. In a few instances where the substituted radicals added considerably to the molecular weight of the compound, an additional amount was used in order to bring the concentration of the sugar fraction of the molecule to the proper figure. Solutions of certain compounds such as gluconic acid and glucosamine hydrochloride, which were distinctly acid, were neutralized with sodium hydroxide solution before filtration. A few additional compounds which were relatively insoluble were added directly to the broth and the whole medium filtered and filled aseptically into sterile tubes. In each instance a period of incubation was allowed, to check the sterility of the medium before it was inoculated.

About twenty-five representative species of the better-known

[1] We are indebted to Dr. W. C. Austin of the Department of Physiological Chemistry of Loyola University for samples of *d*-arabinose, glucoheptose and glucoheptulose.

bacteria and yeasts were employed. Usually two or more strains of each species were included so that well over fifty cultures were used in each of the tests. An incubation temperature of 37° C. was employed for the bacteria and a temperature of 25° to 30° C. for the yeasts. Readings were made daily for the first several days after inoculation and thereafter at definite intervals throughout a period of three weeks. Several controls were included with each test. As a control on the stability of the compound while in solution, two uninoculated tubes were incubated along with the inoculated ones. This served to detect and to eliminate from the tests those compounds which broke down spontaneously with the formation of acid end-products. As a second control, tubes of broth with indicator but without the test sugar were inoculated with each organism. This procedure assisted in the interpretation of results in those instances where an organism was capable of producing slight initial increases in the hydrogen-ion concentration from the peptone or other ingredients of the broth without attacking the sugar.

DISCUSSION OF RESULTS

The results are summarized in tables 1 to 4, where they have been grouped with regard to chemical structure of the compounds. In certain cases it will be noted that the compounds tested do not represent a complete series. Thus, table 1 presents the results with methyl glucoside and methyl mannoside in comparison with glucose and mannose. The preparation of the other glycosides is more difficult and has not yet been completed. We hope to be able to include them in subsequent reports.

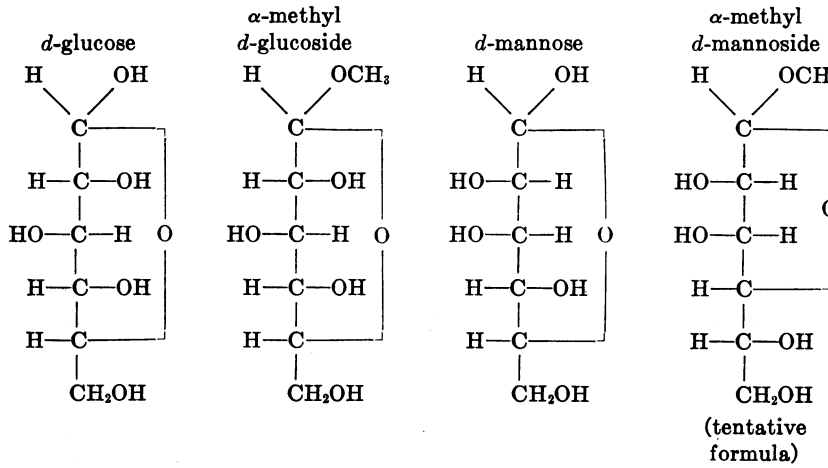
The methyl glycosides

The effect of attaching a methyl radical to two of the common hexoses is shown in table 1. In each instance the methyl radical has been substituted for the hydrogen of the hydroxyl group attached to the number one carbon atom. It is evident that this change in the sugar molecule has brought about a profound difference in the availability of these compounds for use by bacteria. Of the many organisms which fermented glucose and mannose

TABLE 1

Fermentation of methyl glucoside and methyl mannoside compared with glucose and mannose

ORGANISMS	*d*-GLUCOSE	α-METHYL-*d*-GLUCOSIDE	*d*-MANNOSE	α-METHYL-*d*-MANNOSIDE
B. subtilis	A	0	0	0
B. megatherium	A	0	0	0
Bact. prodigiosum	A	0	A	0
Proteus vulgaris	AG	AG	AG	0
Bact. coli	AG	0	AG	0
Bact. aerogenes	AG	AG	AG	0
Bact. friedländeri	A or AG	A or AG	A or AG	0
S. paratyphosum	AG	0	AG	0
S. schottmülleri	AG	0	AG	0
S. aertrycke	AG	0	AG	0
S. enteritidis	AG	0	AG	0
S. cholerae-suis	AG	0	AG	0
E. typhi	A	0	A	0
E. dysenteriae, Flexner	A	0	A	0
E. dysenteriae, Sonne	A	0	A	0
C. diphtheriae	A	0	A	0
C. xerosis	A	0	A	0
Sarcina lutea	0	0	0	0
Staph. aureus	A	0	A	0
Staph. albus	A	0	A	0
Streptococcus (scarlet fever)	A	A*	A	0
Streptococcus (septic sore throat)	A	A*	A	0
Streptococcus (erysipelas)	A	A*	A	0
Pneumococcus, I, II and III	A	A*	A	0
Saccharomyces cerevisiae	AG	AG	AG	0
Torula cremoris	AG	0	AG	0



A = acid to brom-thymol-blue; A* = slow or feeble fermentation; G = gas production; 0 = neither acid nor gas production.

only a few were able to utilize methyl glucoside, while none of them could make use of methyl mannoside.

The difference in behavior of *Bact. coli* and *Bact. aerogenes* in methyl glucoside was found to hold true for a larger number of strains than those shown in the table. This has been reported in detail elsewhere (Koser and Saunders, 1932a). The behavior of the streptococci from scarlet fever, septic sore throat and erysipelas and also of the pneumococci is of interest, since many other organisms, including staphylococci, lacked ability to split the glucoside. The production of acid from methyl glucoside by the streptococci and pneumococci was somewhat slower than when glucose was fermented, and the hydrogen-ion concentration attained was not as high. Although this fermentation was slow it was nevertheless quite distinct after several days, while control cultures inoculated into the same medium without the sugar were uniformly negative. This interesting ability of the streptococci and pneumococci suggests further study of a larger series of these organisms.

The methyl glucoside-splitting abilities of the two yeasts were also of interest. Both of these yeasts ferment glucose readily and, in addition, the Torula ferments lactose with production of acid and gas. *Saccharomyces cerevisiae* was able to break down the glucoside, though it apparently experienced some difficulty in doing so. The production of acid and the evolution of gas were never as pronounced as when glucose itself was fermented. *Torula cremoris*, though able to ferment a wider range of the commoner sugars, was unable to make use of the methyl glucoside.

Alpha-methyl-*d*-mannoside proved to be even more resistant to bacterial attack than the corresponding derivative of glucose. None of the cultures were able to make use of the methyl mannoside. Because of the supposed similarity in structure of these two compounds one might be inclined to expect fermentation by the same organisms which were capable of splitting methyl glucoside, since they do not distinguish between glucose and mannose. Such was not the case however. This brings up the question of the structure of methyl mannoside. Hudson (1930) has maintained that it is a 1–4 ring structure, as shown in table 1, rather than a

1–5 attachment as in the case of the glucoside. Our fermentation tests would seem to bear out Hudson's contention, since if methyl mannoside has the formula shown in the table the results of fermentation tests could conceivably be quite different from those secured with a 1 to 5 attachment. This point will be discussed in more detail in another place.

On reviewing the literature with reference to the use of such compounds as we have employed, one finds comparatively little mention of them. Since the few scattered references deal chiefly with methyl glucoside it seems best to compare our own results with those of other workers at this point. Gruber (1906) reported the use of alpha methyl glucoside, along with a number of the commoner sugars and alcohols, in a study of colon organisms from milk. The glucoside was fermented by some of his "group I" and "group II" cultures. Weintraub (1924) and Hees and Tropp (1926) found that alpha methyl glucoside was fermented by some colon group organisms. The latter authors noted that this substance was used by only a few of their coli cultures and by all (five) of their aerogenes strains. Beta methyl glucoside was fermented by practically all of the coli and aerogenes cultures, though in some instances slowly. Kendall and Gross (1930) tested the effect of 12 microörganisms upon various substituted sugars and related compounds. Alpha methyl glucoside was used by Proteus, *Bact. cloacae*, and *Bact. mucosus-capsulatum*, but not by *Bact. coli* or *Bact. lactis-aerogenes*. Aside from the results with *Bact. aerogenes* our findings are in close agreement with theirs. Lindner (1921) stated that "yeasts" can attack the alpha but not the beta form of methyl glucoside. Our results show that not all yeasts are capable of using the alpha form of this methyl sugar. We have no data in regard to the beta form.

Methyl pentoses

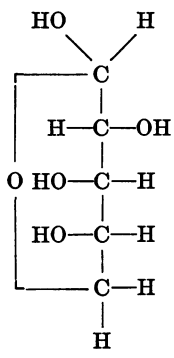
A comparison of *l*-arabinose with its methyl derivative is shown in table 2. Here again it is seen that the insertion of the methyl group, as shown in the structural formula, renders the sugar quite resistant to bacterial attack. The results of fermentation tests with *d*-arabinose are also included in this table. Arabinose is one of

TABLE 2

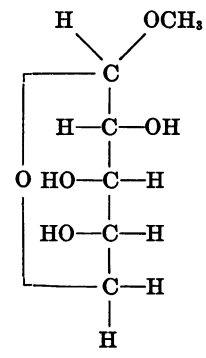
Fermentation of arabinose and methyl arabinoside

ORGANISMS	*l*-ARABINOSE	β-METHYL-*l*-ARABINOSIDE	*d*-ARABINOSE
B. subtilis	0	0	0
B. megatherium	0	0	0
Bact. prodigiosum	0	0	0
Proteus vulgaris	0	0	AG
Bact. coli	AG	0	AG*
Bact. aerogenes	AG	0	AG*
Bact. friedländeri	A or AG	0	A* or AG*
S. paratyphosum	AG	0	AG*
S. schottmülleri	AG	0	AG*
S. aertrycke	AG	0	AG*
S. enteritidis	AG	0	AG*
S. cholerae-suis	0	0	AG*
E. typhi	0	0	0
E. dysenteriae, Flexner	A	0	0
E. dysenteriae, Sonne	A	0	A*
C. diphtheriae	0	0	0
C. xerosis	0	0	0
Sarcina lutea	0	0	0
Staph. aureus	0	0	0
Staph. albus	0	0	0
Streptococcus (scarlet fever)	0	0	0
Streptococcus (septic sore throat)	0	0	0
Streptococcus (erysipelas)	0	0	0
Pneumococcus, I, II and III	0?	0	0
Saccharomyces cerevisiae	0	0	0
Torula cremoris	0	0	0

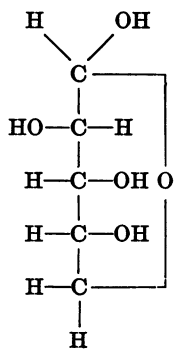
l-arabinose



β-methyl *l*-arabinoside



d-arabinose

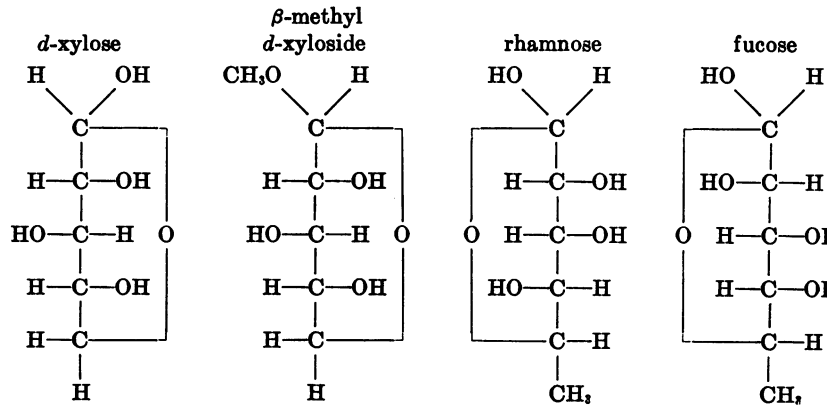


A = acid; A* = slow fermentation; G = gas production.

TABLE 3

Fermentation of xylose compared with methyl xyloside and other methyl pentoses

ORGANISMS	*d*-XYLOSE	β-METHYL-*d*-XYLOSE	RHAMNOSE	FUCOSE
B. subtilis	0	0	0	0
B. megatherium	A	0	0	0
Bact. prodigiosum	0	0	0	0
Proteus vulgaris	0	0	0	0
Bact. coli	AG	0	AG	AG
Bact. aerogenes	AG	0	AG	AG[2]
Bact. friedländeri	A or AG	0	A or AG	A or AG[2]
S. paratyphosum	0	0	AG	AG
S. schottmülleri	AG	0	AG	AG
S. aertrycke	AG	0	AG	AG
S. enteritidis	AG	0	AG	AG
S. cholerae-suis	AG	0	AG	AG
E. typhi	0[1]	0	0	0
E. dysenteriae, Flexner	0	0	0	0?
E. dysenteriae, Sonne	0	0	A	A
C. diphtheriae	0	0	0	0
C. xerosis	0	0	0	0
Sarcina lutea	0	0	0	0
Staph. aureus	0	0	0	0
Staph. albus	0	0	0	0
Streptococcus (scarlet fever)	?	0	0	0
Streptococcus (septic sore throat)	?	0	0	0
Streptococcus (erysipelas)	?	0	0	0
Pneumococcus, I, II and III	?	0	0	0
Saccharomyces cerevisiae	0	0	0	0
Torula cremoris	0	0	0	0



[1] Acid after 2 weeks in *Bact. typhosum* cultures.

[2] One strain of each of aerogenes and friedländer failed to ferment fucose.

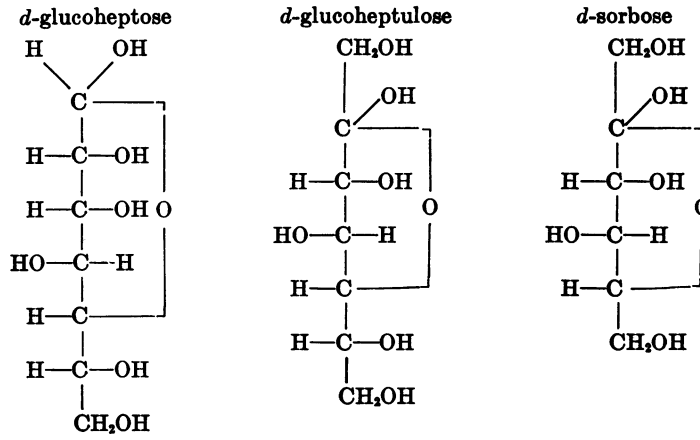
the few sugars where both *d* and *l* forms are available. Therefore a comparison of these two forms, which are exact opposites in chemical configuration and rotation, possesses considerable interest. The most striking result was the slow fermentation of the *d* form by most of the microörganisms. Even such vigorous fermenting types as *Bact. coli* and *Bact. aerogenes* attacked the *d* form with difficulty and usually several days were required for the production of acid and gas. It is noteworthy, however, that two organisms, Proteus and *S. cholerae-suis*, which gave negative results with the *l* form of the sugar, were capable of attacking the *d* form (Koser and Saunders, 1932b).

In further work with other pentoses (table 3) it was found that beta methyl xyloside was not fermented by those bacteria capable of utilizing xylose although a number of these organisms can ferment lactose, showing that they are able to split some beta linkages. Rhamnose and fucose represent compounds with the methyl group replacing a hydrogen atom attached to the fifth carbon.[2] Such a substitution apparently alters the availability of the sugar but little. In this connection it is interesting to compare the spatial configuration and the fermentation of fucose with that of *d*-arabinose.

Heptoses and other miscellaneous compounds

Tests with two seven-carbon sugars, *d*-glucoheptose and *d*-glucoheptulose, were completely negative. A six-carbon sugar, *d*-sorbose, was also found to be quite resistant to bacterial attack. Sorbose presents a configuration similar to that of glucoheptulose but with a shorter side chain. Of all the organisms employed, only a few strains of *Bact. aerogenes* and *Bact. friedländeri* were able to ferment this sugar, a result quite the contrary to that secured with the common hexoses which are fermented readily.

[2] Rhamnose is usually considered as a methyl derivative of the unknown pentose, *l*-lyxose. It will be evident, however, that both rhamnose and fucose may be considered as hexoses with the alcohol group at the sixth carbon atom reduced to CH_3.



The utilization of gluconic acid, glucosamine and a sulphur-containing derivative of glucose is shown in table 4. The change in structure from glucose to gluconic acid prevented the utilization of the molecule by a few organisms, notably the streptococci, pneumococci and yeasts. Our results are in partial agreement with those of Kendall, Bly and Haner (1923), who reported that members of the colon-typhoid-dysentery group, Proteus, the cholera vibrio, and pneumococci were able to make use of gluconic acid, while *Staphylococcus aureus* and *Micrococcus tetragenus* gave negative results.

The substitution of an amino for an hydroxyl group, as in glucosamine, resulted in relatively little change in the availability of the compound insofar as most of the bacteria were concerned. Of the few differences, it will be seen that Proteus made use of glucosamine very sparingly if at all, the yeasts gave completely negative results and the diphtheria bacillus and the streptococci produced a slow fermentation. Glucosamine fermentation by the streptococci and pneumococci and by the diphtheria bacillus was open to question when these organisms were grown in meat-infusion peptone broth. When, however, they were grown in 10 per cent serum broth containing glucosamine, acid was produced promptly.

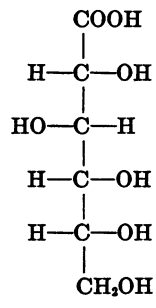
In all of our experiments glucosamine was used as the hydrochloride and neutralized with sodium hydroxide solution. It was

TABLE 4

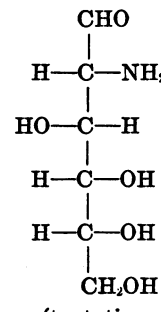
Fermentation of some miscellaneous derivatives of glucose

ORGANISMS	GLUCONIC ACID	GLUCOSAMINE	GLUCOSE ETHYL MERCAPTAL
B. subtilis	0?	A	0
B. megatherium	0	A	0
Bact. prodigiosum	A	A	0
Proteus vulgaris	AG	0 or ?	0
Bact. coli	AG	AG	0
Bact. aerogenes	AG	AG	0
Bact. friedländeri	A or AG	A or AG	
S. paratyphosum	AG	AG	0
S. schottmülleri	AG	AG	0
S. aertrycke	AG	AG	0
S. enteritidis	AG	AG	0
S. cholerae-suis	AG	AG	0
E. typhi	A	A	
E. dysenteriae, Flexner	A	A	
E. dysenteriae, Sonne	A	A	0
C. diphtheriae	A*	A[1]	
C. xerosis	0	0	
Sarcina lutea	0	0	0
Staph. aureus	A*	A	0
Staph. albus	A	A	0
Streptococcus (scarlet fever)	0	A[1]	
Streptococcus (septic sore throat)	0	A[1]	
Streptococcus (erysipelas)	0	A[1]	
Pneumococcus, I, II and III	0	A[1]	
Saccharomyces cerevisiae	0	0	
Torula cremoris	0	0	

gluconic acid

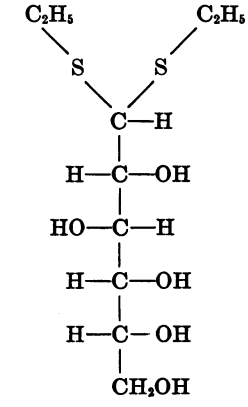


glucos-amine



(tentative formula)

glucose ethyl mercaptal



A* = slight acid production.

[1] Definite fermentation of glucosamine when grown in serum infusion broth.

noticed repeatedly that sterile culture media containing neutralized glucosamine hydrochloride became acid on incubation, changing from pH 7.0–7.1 to 6.0–6.3 within four or five days. This obscured the results somewhat where there was any tendency for an organism to bring about delayed fermentation. However, most organisms fermented the glucosamine promptly and the results were clear cut, since sterile glucosamine broth tubes showed little change in pH within the first twenty-four hours.

A few references on the utilization of glucosamine have appeared in the literature. Meyer (1913) reported that members of the colon-typhoid-dysentery group, with the exception of the Shiga dysentery bacillus, all fermented glucosamine. His results with Proteus, *Staphylococcus aureus* and a *Streptococcus hemolyticus* were recorded as slight or doubtful acid production. Den Dooren de Jong (1926) found that several of the commoner bacteria fermented this compound. Noble and Knacke (1928) studied the action of *Corynebacterium diphtheriae* and related types on glucosamine. Of three strains of the diphtheria bacillus used, only one (Park 8) fermented glucosamine definitely and consistently in all tests. Four of ten diphtheroids fermented glucosamine irregularly, while *C. xerosis* and *C. hofmanni* were consistently negative. On the whole our results agree with those of the foregoing investigators in those instances where a direct comparison can be made.

Glucose ethyl mercaptal was not fermented by any of the organisms used in our series. The tests were somewhat obscured by the bacteriostatic properties of the compound, but all appeared to be negative. When 1.5 per cent glucose ethyl mercaptal was incorporated in nutrient broth, growth was distinctly retarded and only a light turbidity appeared within the first twenty-four hours of incubation, even with the most rapidly-growing types. In the case of some cultures several days were required for production of visible turbidity. Continued incubation of these tests failed to show any evidence of fermentation. Only one reference to the fermentation of glucose ethyl mercaptal has come to our attention. Hees and Tropp (1926) used this compound in their study of colon group organisms. They reported that it was fermented

by "*Bact. lactis aerogenes*" but not by *Bact. coli*. We were unable to confirm this observation.

Acetyl sugars

In addition to the sugars shown in the tables, penta acetyl glucose and tetra acetyl xylose were used in our series of tests. All results with these two acetyl derivatives appeared to be negative, but they have not been given in detail since in some respects the tests were unsatisfactory. Both sugars are only slightly soluble, about 0.2 per cent representing the maximum amount that can be got into solution in broth. Because of the relatively large molecular weight of the acetyl groups, the actual concentration of sugar constituent of the molecule was considerably less than 0.1 per cent. Also, both of these compounds showed a tendency to break down with the liberation of acid products after several days in sterile nutrient broth at 37°C.

SUMMARY

1. Methyl derivatives of several of the commoner sugars, in which the methyl group was substituted for the hydrogen of the hydroxyl group attached to the number one carbon atom, were distinctly resistant to bacterial attack. Thus, alpha-methyl-*d*-glucoside was utilized by comparatively few of the microörganisms which fermented glucose.

2. Similar comparative tests showed that alpha-methyl-*d*-mannoside, beta-methyl-*l*-arabinoside and beta-methyl-*d*-xyloside were not fermented by those organisms capable of breaking down the corresponding unaltered sugars: *d*-mannose, *l*-arabinose and *d*-xylose.

3. The *d*-form of arabinose was fermented much more slowly than the common *l*-form by most bacteria.

4. The methyl pentoses, rhamnose and fucose, in which the methyl group is attached to the fifth carbon atom, were fermented readily.

5. Two sugars containing seven carbon atoms, alpha glucoheptose and alpha glucoheptulose, were not fermented by any of the organisms tested. Glucosamine was used by most of the cultures

which utilized glucose, though in some instances apparently with more difficulty. Gluconic acid was used by many of the bacteria. A sulphur-containing sugar, glucose ethyl mercaptal, gave entirely negative results.

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