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CHEMICAL TRANSFORMATIONS BY ACETOBACTER

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The enzymatic systems elaborated by micro-organisms permit them to catalyze chemical transformations some of which are difficult or impossible to effect by ordinary chemical methods. Although organisms induce chemical changes, the successful application of such enzymatic transformations on a practical scale necessitates, in addition to other factors, the selection of the organism appropriate for the desired reaction and the determination of the optimum conditions. The possible utilization of the fermentation products of the Acetobacter has stimulated interest in the transformations brought about by this group of bacteria. Without going into a detailed discussion, this paper indicates some activities exhibited by this group of micro-organisms. These acetic acid bacteria are motile or non-motile rod-shaped cells, which frequently occur in chains, and have been combined into the genus Acetobacter (1). The present confused state of classification is made apparent by recent studies on the physiological activities of the Acetobacter and a reclassification of this genus is necessary in order to definitely identify the species and thus permit better interpretations of the reported studies.

In 1837 "mother of vinegar" was declared by Kützing (20) to be a living substance which was associated with the conversion of alcohol into vinegar. Twenty-seven years later, in 1864, Pasteur (26) confirmed this opinion and proved that the formation of acetic acid was a physiological process due to the activity of what he assumed to be the single species, *Mycoderma aceti.* Some years later, in 1879, Hansen (15) reported the isolation of three strains of bacteria which caused souring of beer. By the action of one of these newly isolated strains upon glucose, Boutroux (7) obtained what was shown to be gluconic acid. In 1886 Brown (8) isolated another species, *A. xylinum,* which possessed the property of forming thick cellulosic membranes. Bertrand (5) reported some interesting investigations in 1898 which were instigated in an attempt to explain the puzzling failures that occasionally occurred in the preparation of sorbose from the juice of the serb-berry obtained from mountain ash (*Sorbus aucuparia*). He showed that sorbitol, the hexyl alcohol present in the serb-berry, was con-

verted to sorbose (a ketose) by means of a chance contaminant which he called a "sorbose bacterium," later identified as the *A. xylinum*, originally isolated by Brown. Since the early 1900's many other species of acetic acid bacteria have been found, and studies of their activities have been reported by numerous workers; yet only in recent years have serious attempts been made to utilize these bacteria in industries other than the manufacture of vinegar. This very brief paper does not cover the field, hence those further interested in the subject are referred to the recent extensive discussions concerning the biochemistry of the Acetobacter presented by Bernhauer (2) and Butlin (9).

For expediency, the transformations induced by this group of bacteria will be referred to in the following order: (a) action upon alcohols, (b) action upon sugars, (c) action upon acids, (d) synthesis of polysaccharides, and (e) anaerobic dissimilation.

Action Upon Alcohols. The microbial conversion of ethyl alcohol into acetic acid or vinegar is well-known, and such oxidation may be considered typical for the group of primary aliphatic monohydroxy alcohols. It appears that the alcohol is oxidized to the acid through the intermediate step of aldehyde formation. The various species differ in their ability to oxidize the alcohols, as may be noted in table I; hence, proper selection is essential to obtain high yields of the desired end-products. Most of these studies have been made in low concentrations containing about 2-per cent alcohol.

The remarkable ability of the "sorbose bacterium" to oxidize sorbitol led Bertrand (5) to study its behavior toward other polyhydroxy alcohols. Some of these alcohols were utilized as sources of energy and converted to the corresponding ketoses while others did not support growth. As a result of these experiments, Bertrand concluded that alcohols were attacked only if they contained a secondary alcohol group and that the hydroxyl of the secondary group must be on the same side of the chain as the hydroxyl of the following alcohol group according to the Fischer projection. The alcohols of four or more carbons listed in table II have this configuration and yield the indicated ketoses. This conception of Bertrand is universally used as a means to decide the configuration of certain substances.

The importance of research for the selection of an active strain of bacteria and for the determination of optimum conditions of fermentation may be noted from the reports of various investigators since the time of Bertrand's classical work on the formation of sorbose. In the last few years interest in this product has

Table I — Oxidation of aliphatic monohydroxy alcohols by Acetobacter, *giving percentage conversion to product indicated when reported*

Alcohol	Oxidation Product	Name of species — Percentage								
		aceti	*ascendens*	*gluconicum*	*kutzingianum*	*orleanense*	*pasteurianum*	*rancens*	*suboxydans*	*xylinum*
methyl	formic acid								(35)	
ethyl	acetic acid	61	20	43	58	36	41	56	(35)	41
propyl	propionic acid	60	75 (35)	48	43	39	41	29	75 (35)	42
isopropyl	acetone	(4)	(4)			(33)	75 (23)	(35)	(35)	(35)
butyl	butyric acid							(8)	(8)	
amyl	valeric acid				(28)		(28)			

Numbers in ( ) refer to literature cited.
All reference No. 16 except those indicated.

Table II — Oxidation of aliphatic polyhydroxy alcohols by Acetobacter, *giving percentage conversion to product indicated when reported*

Alcohol, showing configuration of hydroxyl groups	Product produced	Name of species — Percentage					
		aceti	*gluconicum*	*melanogenum*	*orleanense*	*suboxydans*	*xylinum*
Ethylene glycol, $CH_2OH - CH_2OH$	glycollic acid	(5)		(35)		(35)	(35)
α-propylene glycol, $CH_3 - \underset{\vert}{C} - CH_2OH$	acetol					70 (9a)	66 (35)
Glycerol, $CH_2OH - \underset{\vert}{C} - CH_2OH$	dihydroxy acetone	90 (16)	(35)	(35)	90 (16)	80 (10)	(5)
Ethrythritol, $CH_2OH - \underset{\vert}{C} - \underset{\vert}{C} - CH_2OH$	erythrulose	(16)	(16)	(8)	(16)	(35)	(5)
Arabitol, $CH_2OH - \underset{\vert}{C} - \underset{\vert}{C} - \underset{\vert}{C} - CH_2OH$	d-xylulose		(14)			(14)	(5)
Sorbitol, $CH_2OH - \underset{\vert}{C} - \underset{\vert}{C} - \underset{\vert}{C} - \underset{\vert}{C} - CH_2OH$	sorbose		79 (16)		10 (16)	(36)	(5)
Mannitol, $CH_2OH - \underset{\vert}{C} - \underset{\vert}{C} - \underset{\vert}{C} - \underset{\vert}{C} - CH_2OH$	frutose	90 (16)	68 (16)	(35)	(16)	(35)	(5)
Perseitol, $CH_2OH - \underset{\vert}{C} - \underset{\vert}{C} - \underset{\vert}{C} - \underset{\vert}{C} - \underset{\vert}{C} - CH_2OH$	perseulose			(35)		100 (33)	(35)

Numbers in ( ) refer to literature cited.

greatly increased, since it is an intermediate for the synthetic production of levo-ascorbic acid or vitamin C (27). In the earlier studies, different species were allowed to act upon 2- to 5-percent solutions of sorbitol for incubation periods varying up to 3 months (3, 5, 35). With the use of a selected organism grown in aerated flasks, Dutch workers (6) reported in 1935 the complete conversion of sorbitol in a 5-percent solution in 3.5 days. The following year, a contribution by Fulmer and his co-workers (10) showed that within 7 to 14 days 80- to 86-percent conversion was possible by surface fermentations in solutions containing 15- to 35-per cent sorbitol. More recently with the use of equipment designed for fermentation studies under pressure, aeration, and agitation (12, 17, 37), installed in the laboratories of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture at Washington, D. C. and Ames, Iowa, quantitative conversion of sorbitol to sorbose was obtained within 13.5 to 45 hours in 10- to 30-per cent solutions (36, 38).

Although sorbose formation from sorbitol appears to hold the center of interest at present, studies have been made on the oxidation of other alcohols, and good yields have been reported for the production of acetol from propylene glycol (9a), dihydroxyacetone from glycerol (34), levulose from mannitol (11), and perseulose from perseitol (33).

Action Upon Sugars. The results reported in table III on the bacterial production of gluconic acid make it evident that in addi-

Table III — Oxidation of glucose by Acetobacter *to the acids indicated*

Acids	Name of species
gluconic alone	aceti (7) xylinum (8) suboxydans (9a)
gluconic + 5-keto gluconic	rancens (35) suboxydans (35) xylinum (5)
gluconic + 5-keto gluconic + acetic	aceti (16) gluconicum (16) xylinum (16)
gluconic + acetic	pasteurianum (16) rancens (16)
gluconic + lactic + acetic + formic	xylinum (13)
gluconic + succinic	other species (30)

Numbers in ( ) refer to literature cited.

tion to other conditions, proper selection of the organism is essential. Studies in progress in our laboratory with 14 strains of Acetobacter show that, with aeration, some strains can convert about 90 per cent of the available glucose to gluconic acid within 96 hours in a 5-per cent glucose solution.

Other sugars that have been oxidized and their products of

oxidation are xylose to xylonic acid; arabinose to arabonic acid (35); galactose to galactonic acid (16); and fructose to keto-gluconic, acetic (16), and kojic acids (31).

Action Upon Acids. The ability of these bacteria to oxidize acids has been indicated by the production of keto-gluconic acids from glucose, evidently through the intermediate formation of gluconic acid. However, a summary of the literature made by Butlin (9) concerning the action on the aliphatic acids shows that little is known concerning the end-products. In general, the decomposition of keto- and hydroxy-acids produces an acid with one less carbon atom. It is possible that polyhydroxy acids other than gluconic may be converted to keto acids by dehydrogenation. A Japanese worker, Miyaji (21), has reported the action of 10 strains of Acetobacter upon six amino acids. Some of the organisms caused deamination, such as the conversion of glycine to acetic acid; some replaced the amino group by a hydroxyl, as in the conversion of l-leucine to l-isopropyllactic acid; while others caused decarboxylation in addition to other reactions, as in the formation of succinic acid from d-glutamic acid.

Synthesis of Polysaccharides. Reference has been made to Brown's (8) observations on the formation of cellulose by *A. xylinum*. Later it was shown that this cellulose was identical with that found in cotton (18) and that the amount formed depends upon the substrate (19, 32). According to our observations, *A. melanogenum* produces a gum-like substance soluble in water and insoluble in alcoholic solutions. The mucilaginous capsules of *A. pasteurianum* and *A. kutzingianum* also contain polysaccharides which stain blue with iodine (9).

Anaerobic Dissimilation. In 1928 it was shown that appreciable amounts of alcohol and carbon dioxide were formed when the aerobic *A. ascendens* acted upon glucose under anaerobic conditions (24). This result was amplified using other species of the acetic acid bacteria (29). Just as acetaldehyde in very dilute solutions is converted to ethyl alcohol and acetic acid (25), so are other

Table IV — Some anaerobic dissimilations by Acetobacter

Substrate	Products formed
glucose	ethanol, CO_2 (29)
pyruvic acid	acetaldehyde, CO_2 (29)
acetaldehyde	acetic acid, ethanol (25)
n-butaldehyde	butanol, butyric acid (25)
iso-valeraldehyde	iso-valeric acid, iso-butylcarbinol (25)
propaldehyde	n-propanol, propionic acid (22)
furfural	pyromucic acid, furfuryl alcohol (22)

Numbers in ( ) refer to literature cited.

aldehydes dismuted to their respective alcohols and acids (*22*). Hypotheses explaning these anaerobic breakdowns have been suggested by the various authors (*22*, *24*, *25*, *29*). Table IV presents some of the dissimilation products of various substrates.

SUMMARY

A brief survey of the literature concerning the activities of the Acetobacter has been given in order to indicate the importance of this group of bacteria. Many products may be obtained through the activity of these organisms upon various substrates. Some of these products of fermentation are finding practical applications, while others are under investigation. Application of these Acetobacter for efficient catalytic transformations necessitates the selection of a specific strain and the establishment of conditions optimum for the desired transformations.

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