SYNTHESIS OF CELLULOSE BY ACETOBACTER XYLINUM

VI. GROWTH ON CITRIC ACID-CYCLE INTERMEDIATES

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ABSTRACT

GROMET-ELHANAN, ZIPPORA (The Hebrew University, Jerusalem, Israel) AND SHLOMO HESTRIN. Synthesis of cellulose by Acetobacter xylinum. VI. Growth on citric acid-cycle intermediates. J. Bacteriol. 85:284-292. 1963.—Acetobacter xylinum could be made to grow on ethanol, acetate, succinate, or L-malate. The growth was accompanied by formation of opaque leathery pellicles on the surface of the growth medium. These pellicles were identified as cellulose on the basis of their chemical properties, solubility behavior, and infrared absorption spectra. Washed-cell suspensions prepared from cultures grown on ethanol or the organic acids, in contrast to washed sugar-grown cells, were able to transform citric-cycle intermediates into cellulose. The variations in the substrate spectrum of cellulose synthesis between sugar-grown cells and organic acids-grown cells were found to be correlated with differences in the oxidative capacity of the cells. The significance of the findings that A. xylinum could be made to grow on ethanol on complex as well as synthetic media is discussed from the viewpoint of the whole pattern of Acetobacter classification.

Acetobacter is particularly prone to mutation, even in characters which have been used for species classification (Shimwell, 1956, 1957, 1959; see, however, De Ley, 1961). Of the five biochemical criteria established by Frateur (1950) for the classification of acetic acid bacteria, four (catalase activity, ketogenic power, production of acid from glucose, and ability to synthesize cellulose) were shown by Shimwell to be highly mutable. On the other hand, behavior in respect to the fifth criterion (growth on Hoyer's synthetic medium in which ammonium and ethanol serve as sole nitrogen and carbon sources) was found to be stable (Shimwell, 1957b, 1959; Shimwell and Carr, 1961).

Strains of Acetobacter, which are Hoyer-negative but positive for the other four criteria established by Frateur, have been classified as Acetobacter xylinum. Previous attempts to grow A. xylinum on ethanol or acetate, in either synthetic or complex media, have been unsuccessful (Hoyer, 1988; Beijerinck, 1898; Tosic and Walker, 1946; Frateur, 1950; Rao and Stokes, 1953; Hall et al., 1956; Shimwell, 1957a) except in one case, that of A. xylinum var. africans NCIB 7029 (Hall et al., 1956; Steel and Walker, 1957).

In sugar-grown A. xylinum, it was shown that carbohydrates are oxidized by way of a pentose cycle (Gromet, Schramm, and Hestrin, 1957), and that organic acids are oxidized by way of a citric cycle which also participates in the sugar oxidation (Gromet-Elhanan, 1960a). Suspensions of washed sugar-grown cells of this species formed cellulose from sugars and related substrates via the pentose cycle, but formed no cellulose from ethanol or any citric-cycle intermediate (Schramm, Gromet, and Hestrin, 1957a). Moreover, A. xylinum failed to form C14-cellulose even when this organism was grown on C14-acetate or C14-ethanol together with glucose (Greathouse, Shirk, and Minor, 1954). However, Bourne and Weigel (1954) demonstrated synthesis of C14-cellulose from C14-lactate by A. acetigenum (Henneberg, 1898) growing in a medium which contained some glucose, and Stacey (1954) mentioned in a review that A. acetigenum also forms C14-cellulose in a medium which contains C14-acetate and glucose. The findings thus suggest that carbon can flow in sugar-grown A. xylinum cells from the pentose cycle into the citric cycle but that flow in the reverse direction is blocked, whereas in A. acetigenum this block either does

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not occur or an alternative pathway for cellulose synthesis has been acquired.

It remains to be ascertained whether growth of *A. xylinum* in the presence of citric-cycle intermediates but in the absence of glucose can yield cells which are able to transform citric-cycle intermediates into cellulose. In the present paper, it is shown that, under appropriate conditions, *A. xylinum* can in fact be made to grow on citric-cycle intermediates or on ethanol or acetate; that such growth is accompanied by formation of a cellulose pellicle; and that washed-cell suspensions prepared from such cultures transform citric-cycle intermediates into cellulose. A preliminary account of this work has been published (Gromet-Elhanan, 1960b).

**MATERIALS AND METHODS**

The strain of *A. xylinum* was the same as that employed in earlier investigations (Hestrin and Schramm, 1954; Schramm and Hestrin, 1954a).

**Composition of culture media.** The pH levels were adjusted as necessary by addition of HCl or NaOH. The media were as follows (composition in per cent, w/v): (A) Substrate (glucose, fructose), 2; yeast extract (Difco), 0.5; peptone (Difco), 0.5; dipotassium phosphate, 0.1; pH 7.0. For experiments at pH 5.0, the monopotassium salt was used. (B) Substrate (ethanol, acetate, succinate, l-malate, or citrate), 2; yeast extract (Difco), 0.4; peptone (Difco), 0.3; L-asparagine, 0.1; monopotassium phosphate, 0.3; pH 5.0. (C) Substrate (succinate, l-malate, or citrate), 2; yeast extract (Difco), 0.5; peptone (Difco), 0.5; monopotassium phosphate, 0.3; pH 4.0. (D) Substrate (ethanol, acetate, or citrate), 2; yeast extract (Difco), 1.0; peptone (Difco), 0.5; monopotassium phosphate, 0.3; pH 4.0 or 5.0, as needed. (E) As D, but with addition of 0.1% L-malate; pH 4.0. (F) Hoyer's synthetic medium (2% glucose as carbon source) prepared according to Frateur (1950). (G) As F, but with 2% ethanol as carbon source. (H) As G, but with addition of 0.1% L-malate.

Transfers from glucose to a nonsugar medium were made in B. Growth in this medium was slow except with succinate. At subsequent transfers from B to either C or D, a rapid growth was achieved. In the case of acetate, the growth was also slow in D (Table 1), but was rapid in E, which was therefore always used for the mass culture on acetate.

**Effect of pH on growth.** The effect of the initial pH of the growth medium on the cell yield merits comment. Growth of *A. xylinum* was found to be inhibited at pH > 7.0. Elsewhere, it has been reported that growth of *A. xylinum* is inhibited at pH < 3.5 (Tosic and Walker, 1946). Accordingly, with acid-generating substrates, such as glucose, optimal growth was obtained when the initial pH of the medium was held within 5.0 to 7.0. With alkali-generating substrates of the type of a salt of an organic acid, eventual rise of the pH beyond 7.0 could not be completely prevented but was sufficiently delayed (> 60 days) when the initial pH was brought to 4.0. Some increase of pH levels also occurred during growth in media containing fructose or ethanol as the carbon source, but could be held within desired limits by setting the initial pH value at 5.0. The extent of the variation of pH levels during growth in the presence of different substrates at selected initial pH values is shown in Fig. 1.

**Culture conditions.** Growth on substrates was examined in test tubes containing 5 ml of medium held at 30 C under static conditions. Growth was manifested by formation of a pellicle.

![FIG. 1. Changes of pH with time, in cultures of *Acetobacter xylinum* on different carbon sources.](http://jb.asm.org/)
Mass cultures were grown at 30°C in a shallow layer of quiescent liquid contained in an aluminum pan under loose cover. The inoculum was prepared as follows. About 1.5 ml of culture stock in a test tube were transferred to 100 ml of medium incubated in a Roux flask (layer thickness of medium, 1.0 cm) for 3 days at 30°C. From this flask, 10 ml were then transferred to 100 ml of fresh medium in a second flask which was incubated for 2 days at 30°C and then added to the mass medium in a ratio of 1:10. The cells were harvested at 38 hr, but with acetate the crop was harvested at 60 hr.

Preparation of washed-cell suspensions. Cells were prepared as described previously (Hestrin and Schramm, 1954), but were washed twice in citrate phosphate buffer, pH 5.0 (0.032 M phosphate; 0.16 M citrate), and twice in distilled water. All operations were carried out in the cold. Cells were used immediately after preparation.

Test systems. To examine the effect of agitation, cultures were grown in 100-ml Erlenmeyer flasks containing 30 ml of medium swirled in a Rose-Kershaw shaking apparatus (A. H. Thomas Co., Philadelphia, Pa.) maintained at 30°C.

Conventional Warburg techniques were used in all manometric determinations, CO₂ being absorbed by alkali in the central cup. The substrate was always put in the side arm and added to the reaction mixture after 10 min of equilibration at 30°C. The gas phase was air and the total volume was 2 ml. Endogenous readings represented <10% of the total readings. Values are reported after having been corrected for endogenous rates.

Cellulose synthesis was tested in a system (10 ml) consisting of 30 mg (dry wt) of cells and 10 mg of substrate in potassium phosphate buffer (pH 6.5; 0.025 M phosphate) contained in stoppered 125-ml Erlenmeyer flasks shaken for 5 hr at 100 oscillations/min in a water bath at 30°C. Except as otherwise stated, the gas phase was O₂. Synthesis of cellulose was stopped by flooding the mixture with ether. The cellulose formed and the enmeshed cells were then sedimented by centrifugation, and the precipitate was repeatedly washed. The cellulose initially present (never exceeding 0.2 mg) was deducted from the total amount found after incubation of cells with substrate.

Determination of infrared absorption spectra. The pellets produced under static growth conditions in 100 ml of medium of a Roux flask were used for optical analysis. In one case, an analysis was also made on a pellet produced by washed cells in a system (20 ml) consisting of 27 mg (dry wt) of ethanol-grown cells and 200 mg of glucose in potassium phosphate buffer (pH 6.5; 0.025 M phosphate) contained in a petri dish and incubated at 30°C for 20 hr. The pellets were washed in water. Proteins were removed from the pellets by treatment with 4% NaOH at 30°C for 3 days under N₂, and then for 10 min at 100°C in air. Transparent pellets obtained by these means were then washed twice in dilute acetic acid and twice in water and finally lyophilized (4 hr) while spread out on petri dishes, to afford thin films which were analyzed with the procedure of Forziati and Rowen (1951) in a double-beam infrared recording spectrophotometer (Baird Ltd.), kindly placed at our disposal by the Department of Organic Chemistry, Hebrew University.

Analytical methods. Cellulose was determined essentially by the method of Schramm and Hestrin (1954b), except that the tedious neutralization step was circumvented by the use of a phenol-sulphuric acid reagent (Dubois et al., 1956) to measure the glucose released by acetyl-sis-hydrolysis from cellulose. Values for cellulose obtained with this modification and by the original method of hexose determination using a copper reagent were identical. Reducing power was estimated with Somogyi's copper reagent (Nelson, 1944), fructose with resorcyl (Roe, Epstein, and Goldstein, 1949), and glucose (as aldose) by a microadaptation of an iodimetric method (Macleod and Robison, 1929).

The radioactivity of C¹⁴ was measured in a thin-window Geiger-Müller tube. Substrate solutions were put on thin lens paper discs together with 0.5 ml of 0.2% agar, dried in planchets, and counted. Cellulose samples were washed, freed from protein, then washed again and homogenized in a small amount of water, collected on Millipore filter discs, dried in planchets, and counted. Counts were corrected for self-absorption.

Substrates. Radioactive fructose T-C¹⁴ was obtained from the Radiochemical Centre, Amersham, England. Acetate 1-C¹⁴ was from Research Specialties Co., Berkeley, Calif.
SYNTHESIS OF CELLULOSE BY A. XYLINUM

Results

Growth of A. xylinum on ethanol and some organic acids. Conditions enabling rapid pellicle formation by A. xylinum growing on different substrates are summarized in Table 1. It can be seen that A. xylinum could be made to grow on ethanol, acetate, succinate, and L-malate. Under static conditions, growth was always indicated by formation of a pellicle on the surface of the liquid medium. The medium beneath the pellicle remained transparent with no diffuse growth. Transfer from static to agitated conditions caused appearance of diffuse turbid growth accompanied by small cellulose bodies. But, as expected from the work of Schramm and Hestrin (1954a), no cellulose was formed after a few transfers under agitation, growth being indicated only by turbidity. Transfer from such turbid cultures to test tubes kept under static conditions gave no pellicles, only diffuse growth with a precipitate at the bottom.

Growth on ethanol was obtained at the first transfer from a glucose-grown culture. At a second transfer on ethanol, a pellicle was formed in only one of four test tubes, but thereafter all further transfers in ethanol from this positive tube gave pellicle formation. In this case, the ability to form cellulose from ethanol has obviously been acquired, and the results cannot be attributed to traces of glucose in the medium (see Steel and Walker, 1957).

Growth on acetate did not appear at the first transfer from either glucose- or ethanol-grown cultures. After many unsuccessful attempts to grow A. xylinum on acetate, a pellicle appeared in one test tube. All subsequent transfers from this tube to acetate medium gave pellicle formation.

With succinate and L-malate as carbon sources, pellicle formation was observed at the first transfer from either glucose- or ethanol-grown cultures. With citrate, on the other hand, only a very fine and incoherent membrane appeared on medium B, C, or D at pH values in the range of 3.5 to 7.0 after 7 days of incubation; it showed no further increase in mass with time.

Growth of A. xylinum on Hoyer's medium. The ability to grow on ethanol and acetate could be due neither to simultaneous mass adaptation nor to selection of a pre-existing variant, since it appeared only in one of many cultures examined, and was retained even after five transfers on glucose. It was therefore interesting to ascertain whether these ethanol-grown cells were also able to grow on Hoyer's synthetic medium. These cells could be grown readily on Hoyer's medium (Table 1) when glucose served as the carbon source (medium F). The classical Hoyer's medium with ethanol as substrate (medium G) gave a fragile transparent sac at the bottom of the test tube, which never rose to form the characteristic coherent opaque pellicle at the surface. However, addition of a small amount of L-malate to this medium permitted the production of a normal pellicle. The added small amount of L-malate gave no pellicle in the absence of ethanol. Also, after shaking down the pellicle formed in ethanol and L-malate (medium H), another pellicle appeared; this process could be continued through the formation of three to four pellicles in the same test tube.

On the basis of these results, ethanol-grown A. xylinum cells could be regarded as fulfilling all of Frateur's biochemical criteria used in Acetobacter classification. Another example of an Acetobacter positive for all of Frateur's criteria was found by Carr (1958). He isolated, from

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Designation</th>
<th>pH</th>
<th>Timea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>A</td>
<td>5.0</td>
<td>2</td>
</tr>
<tr>
<td>Ethanol</td>
<td>D</td>
<td>5.0</td>
<td>3</td>
</tr>
<tr>
<td>Acetate</td>
<td>D</td>
<td>4.0</td>
<td>7</td>
</tr>
<tr>
<td>Succinate</td>
<td>C</td>
<td>4.0</td>
<td>3</td>
</tr>
<tr>
<td>L-Malate</td>
<td>C</td>
<td>4.0</td>
<td>3</td>
</tr>
<tr>
<td>Glucose</td>
<td>F</td>
<td>5.6</td>
<td>5</td>
</tr>
<tr>
<td>Ethanol</td>
<td>G</td>
<td>5.6</td>
<td>±d</td>
</tr>
<tr>
<td>Ethanol</td>
<td>H</td>
<td>5.6</td>
<td>10</td>
</tr>
</tbody>
</table>

a Days after inoculation required for formation of a pellicle of 0.3-cm thickness.

b The same rapid cellulose formation was also observed at an initial pH of 7.0.

c With glucose, the pellicle continued to thicken to 1.5 cm. Pellicles on other substrates stopped growing after 14 days, at a thickness of 0.5 cm.
d A fragile sac appeared at the bottom of these tubes after 7 days (see text).
ciders, a strain of *A. acetii* which gave a positive cellulose reaction according to Frateur's tests, although no characteristic cellulose pellicle was formed.

**Identification of cellulose in pellicles produced on ethanol and succinate.** The pellicles formed during growth of *A. xylinum* on ethanol, acetate, and succinate were thinner than those produced on glucose but in other respects were similar in appearance to the latter. The pellicles were insoluble in hot 4% NaOH, and afforded products which reduced copper reagents and iodine after acetylation and hydrolysis according to Schramm and Hestrin (1954b). Unambiguous identification of cellulose in pellicles produced by ethanol and succinate cultures was made on the basis of the infrared absorption spectrum (Fig. 2). The spectra obtained were identical with the spectrum of a pellicle produced on glucose. This cellulose has been identified as high-polymer, native, crystalline α-cellulose (Shirk and Greathouse, 1952; Hestrin and Schramm, 1954). No pellicle was formed in the nutrient medium when the carbon source was omitted. It is highly likely, therefore, that cultures grown on ethanol or succinate synthesized cellulose from these substrates.

**Cellulose synthesis by washed-cell suspensions.** Further proof that the organic acids acetate, pyruvate, succinate, and L-malate can serve as substrates for cellulose synthesis was obtained by work with washed-cell suspensions prepared from cultures grown on ethanol, acetate, or succinate. The washed cells were found capable of cellulose synthesis in reaction mixtures containing only buffer and substrate. In each case, the synthesized polymer was identified as cellulose on the basis of its chemical properties and solubility behavior. No pellicle thick enough for measuring infrared absorption could be obtained by the action of these washed cells on the organic acids. However, a pellicle produced from glucose by ethanol-grown washed cells was found to have the same absorption spectrum as that of the pellicles formed in cultures (Fig. 2). It follows, therefore, that such washed cells are capable of cellulose synthesis.

Washed succinate-grown cells afforded cellulose from glucose, succinate, and pyruvate (1.2, 0.4, and 0.4 mg, respectively, from 10 mg of substrate). Addition of pyruvate to a reaction mixture with glucose afforded the formation of 1.6 mg of cellulose. Synthesis from pyruvate was totally inhibited by fluoroacetate at a concentra-
tion (10⁻² M) which inhibited oxidation of pyruvate. This is in contrast to the case with glucose, in which even 5 x 10⁻⁴ M fluoroacetate did not completely suppress cellulose synthesis (Gromet-Elhanan, 1960a).

Synthesis experiments with washed ethanol-grown cells are summarized in Tables 2 and 3. Cellulose was formed from glucose, fructose, L-malate, and pyruvate, but not from ethanol or acetate, even in the presence of an added small amount of L-malate.

The effect of added pyruvate on cellulose synthesis from sugars was tested in mixtures of glucose or fructose and pyruvate (Table 3). In sugar-grown cells, synthesis from glucose and fructose was found to be inhibited by pyruvate and acetate (Schramm et al., 1957a). However, in A. xylinum cultures grown on mixtures of glucose and ethanol or acetate, these adjuncts, though not affording cellulose, stimulated cellulose synthesis from glucose (Minor et al., 1954; Greathouse et al., 1954). In ethanol-grown cells as in succinate-grown cells, under conditions in which pyruvate by itself afforded cellulose, the addition of pyruvate to glucose did not significantly affect the cellulose synthesis. However, when pyruvate was added to fructose, the amount of cellulose formed greatly exceeded the sum of the amounts which these substrates gave separately. This increase in cellulose was found to be due to the stimulation by pyruvate of cellulose synthesis from fructose, since in the absence and presence of pyruvate the radioactivity recovered in cellulose amounted to 16 and 42%, respectively, of the fructose metabolized. On the other hand, the rate of sugar consumption was not altered in the presence of pyruvate, as can be seen from the chemical and isotopic data in Table 3. Also, the synthesis from pyruvate itself was unaltered in the presence of fructose.

The observed different stimulatory effects on glucose and fructose can be explained by differences in their metabolic patterns: more than 95% of the glucose was consumed during the reaction as compared with about 42% of the fructose (Tables 2 and 3). The reason for this difference is the fact that the cells used here stemmed originally from cells grown on glucose at an initial pH 7.0. These glucose-grown cells were adapted to fructose because of the small amount of fructose formed from glucose when autoclaved at pH 7.0, but the transfers on ethanol in the total absence of fructose caused partial deadaptation.

Washed acetate-grown cells synthesized cellulose from glucose, fructose, succinate, and pyruvate. Only traces of cellulose were formed from acetate, but the addition of a small amount of glucose raised the synthesis to a level which significantly exceeded that found with either substrate alone (Table 4). With C¹⁴-acetate in the

### Table 2. Cellulose synthesis by washed ethanol-grown cells

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Production of cellulose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>2.0</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.3</td>
</tr>
<tr>
<td>L-Malate</td>
<td>0.5</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.4</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.0</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Table 3. Effect of pyruvic acid on cellulose synthesis from fructose and glucose by ethanol-grown cells

<table>
<thead>
<tr>
<th>Test system</th>
<th>Amount of sugar metabolized (μmoles)</th>
<th>Production of cellulose as fraction of</th>
<th>Sugar metabolized (μmoles)</th>
<th>Sugar metabolized (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>52.6 94</td>
<td>6.1</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>22.5 40</td>
<td>5.0</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>Pyruvate</td>
<td></td>
<td>0.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glucose + Pyruvate</td>
<td>53.0 95</td>
<td>6.4</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>Fructose + Pyruvate</td>
<td>23.6 42</td>
<td>11.4</td>
<td>45.8</td>
<td></td>
</tr>
</tbody>
</table>

a Fructose T-C¹⁴ (380,000 counts/min) was used in these experiments. In the absence and presence of pyruvate, the decrease in soluble C¹⁴ in the medium amounted to 140,000 and 150,000 counts/min, respectively, whereas 24,000 and 55,000 counts/min were recovered in cellulose.

b Amount added: 10 mg.

c Calculated on the difference: total cellulose minus cellulose formed with pyruvic acid alone.
presence of glucose, the recovery of counts in cellulose corresponded to 1.1% of the total C\(^{14}\). On the basis of this result, it could be concluded that cellulose synthesis from acetate was not affected by the presence of glucose, and that the higher yield of cellulose observed in the presence of added glucose was due to stimulation of cellulose synthesis from glucose by the acetate.

From the foregoing observations, it is evident that, in \textit{A. xylinum} cells able to form cellulose from acetate or pyruvate, these acids could stimulate cellulose synthesis from sugars, but only when the sugar metabolism proceeded at a low rate or when small amounts of sugar were used. The acids did not affect the rate of sugar consumption, and under these conditions the energy formed by oxidation of part of the sugar might not be enough to maintain synthesis at its maximal rate. The stimulation might then be explained by a sparing mechanism whereby the acids are preferentially oxidized, thus sparing the sugar substrate for cellulose synthesis.

**Oxidation of substrates by cells grown on ethanol, acetate, or glucose.** In view of the demonstrated effects of the carbon source in the growth medium on the substrate spectrum of cellulose synthesis, it was of interest to learn whether the observed variations in synthetic ability were correlated with differences in the oxidative capacity of the cells. The results (Table 5) indicate that growth on glucose resulted, in fact, in loss of ability to oxidize acetate and in a delay in the onset of oxidation of dicarboxylic acids, which were not attacked at all during the first 20 min. Ethanol-grown cells occupied, in these respects, an intermediate position between that of the glucose and the acetate cells.

**DISCUSSION**

The experiments here reported demonstrate the possibility of obtaining a mutant (in Shimwell’s definition: Shimwell, 1959) of \textit{A. xylinum} which could be grown on Hoyer’s medium. Hence, even this biochemical criterion, which was found to be stable by Shimwell (1957b, 1959), is in fact mutable. De Ley (1961) did not accept Shimwell’s view of the abnormally high mutability of the acetic acid bacteria, since most of his strains showed “unchanged sets of properties over a period of three years.” However, De Ley and Stouthamer (1959) observed a spontaneous loss of pigment formation (change of \textit{Glucconobacter} (\textit{Acetobacter}) \textit{melanogenum} into \textit{G. suboxydans}). Tosic and Walker (1946) reported on the spontaneous loss and gain of the ability of \textit{A. kuetzingianum} to form a leathery (cellulose,

**Table 5. Oxidation of citric-cycle intermediates by Acetobacter xylinum cells grown on various substrates**

<table>
<thead>
<tr>
<th>Substrates of oxidation</th>
<th>Carbon source in growth medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose(^a)</td>
</tr>
<tr>
<td></td>
<td>(\text{O}_2)</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>14.0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>9.0</td>
</tr>
<tr>
<td>Acetate</td>
<td>0</td>
</tr>
<tr>
<td>Succinate</td>
<td>1.9</td>
</tr>
<tr>
<td>Fumarate</td>
<td>1.3</td>
</tr>
<tr>
<td>L-Malate</td>
<td>3.1</td>
</tr>
<tr>
<td>(\alpha)-Ketoglutarate</td>
<td>0.8</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.3</td>
</tr>
</tbody>
</table>

\(^a\) Rates are expressed relative to glucose = 10, for the time interval 0 to 30 min.

\(^b\) Mixture contained 8 mg of lyophilized cells, 10 \(\mu\)moles of substrate, and 100 \(\mu\)moles of sodium cacodylate; pH 5.9.

\(^c\) Mixture contained 2 mg (dry wt) of fresh cells, 10 \(\mu\)moles of substrate, and 100 \(\mu\)moles of potassium phosphate; pH 6.0.

\(^d\) Mixture contained 6 mg (dry wt) of fresh cells, 15 \(\mu\)moles of substrate, and 50 \(\mu\)moles of sodium cacodylate; pH 5.5.

\(\text{mg}\) = milligrams.

**Table 4. Cellulose synthesis by washed acetate-grown cells**

<table>
<thead>
<tr>
<th>Test system</th>
<th>Production of cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>Supplement</td>
</tr>
<tr>
<td>Glucose</td>
<td>---</td>
</tr>
<tr>
<td>Fructose</td>
<td>---</td>
</tr>
<tr>
<td>Succinate</td>
<td>---</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>---</td>
</tr>
<tr>
<td>Acetate</td>
<td>---</td>
</tr>
<tr>
<td>Acetate(^*)</td>
<td>Glucose(^a)</td>
</tr>
<tr>
<td>---</td>
<td>Glucose(^b)</td>
</tr>
</tbody>
</table>

\(^*\) Acetate 1-C\(^{14}\) (126,000 counts/min) was used in this experiment; 1,273 counts/min were recovered in cellulose.

\(^a\) Amount added: 1 mg.
see Kaushal and Walker, 1951) pellicle. Kulka and Walker (1954) observed a spontaneous gain in ability to produce gluconic acid from glucose by A. ascendens (changes into A. rancens), and Schramm and Hestrin (1954a) obtained cellulose-less mutants of A. xylinum (changes into A. mesozydans). Also, new isolates corresponding to certain species, but differing in one biochemical property, were reported by Frateur and Simonart (1952), who isolated catalase-negative A. rancens and A. mesozydans, and by Carr (1958), isolating a cellulose-producing A. aceti.

On the basis of the foregoing observations, all the biochemical criteria used in Acetobacter classification (Frateur, 1950; Breed, Murray, and Smith, 1957) seem to be mutable. Shimwell (1957b) and De Ley (1961) pointed out that Acetobacter species exhibit a stepwise gradation in their biochemical properties, with no abrupt change between neighboring species. It follows, therefore, that even one mutation might be enough to shift one species into another, or cause the appearance of new species, as indicated above. Therefore, the demonstrated mutability of all the biochemical criteria, whether high or low, leads one to accept Shimwell’s conclusion that the acetic acid bacteria cannot be classified at all (Shimwell, 1959).

Growth of A. xylinum on ethanol, acetate, and succinate was accompanied by formation of a cellulose pellicle. Moreover, washed-cell suspensions prepared from such cultures transformed citric-cycle intermediates into cellulose. Results obtained in sugar-grown cells, from experiments with specifically labeled hexoses, suggested that cellulose arises in A. xylinum; as in green plants, from an intracellular pool of hexose phosphate, formed by way of a pentose cycle (Schramm, Gromet, and Hestrin, 1957b). If this postulate is applied also to nonsugar-grown cells, then cellulose synthesis from citric acid-cycle intermediates would require formation of hexose phosphate from these intermediates. Possible pathways for cellulose synthesis from organic acids by succinate-grown A. xylinum cells are discussed by Beniziman and Burger-Rachamimov (1962).

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LITERATURE CITED


