2-KETOGLUCONATE FERMENTATION BY STREPTOCOCCUS FAECALIS

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ABSTRACT

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2-Ketogluconate fermentation by Streptococcus faecalis. J. Bacteriol. 87:844-851. 1964.—Streptococcus faecalis 10Cl did not grow with 2-ketogluconate alone as an energy source, but did grow when gluconate was added. More growth was obtained than could be accounted for by the gluconate alone. The requirement for gluconate in the stimulation of growth on 2-ketogluconate was found to be stochiometrically, not catalytic. Glucose did not replace gluconate in this phenomenon, apparently owing to the repression of the 2-ketogluconate pathway by glucose. Resting cells grown on a combination of gluconate and 2-ketogluconate did ferment 2-ketogluconate without added gluconate. Fermentation balance studies with resting cells detected the following products in moles (per mole of 2-ketogluconate): carbon dioxide, 0.98; lactic acid, 0.19; formic acid, 1.42; acetic acid, 0.70; and ethanol, 0.42. 2-Ketogluconate-1-C14 and -2-C14 were prepared and fermented. The data were interpreted to show that 90% of the substrate was decarboxylated to carbon dioxide and pentose phosphate. Pentose phosphate was then fermented to pyruvate through the sedoheptulose diphosphate variation of the pentose phosphate pathway found in this organism. The other 10% of the substrate was converted to pyruvate by way of the Entner-Doudoroff pathway. Calculations of the energy available by the above combination of pathways indicated that about 2.3 moles of adenosine triphosphate per mole of 2-ketogluconate could be obtained if the energy available in acetate formation is conserved through the acetokinase reaction.

It was previously reported that Streptococcus faecalis 10CI grown on gluconate was able to ferment 2-ketogluconate (Sokatch and Gunsalus, 1957). 2-Ketogluconate was subsequently ruled out as an intermediate in gluconate fermentation because aged or dried cells lost the ability to ferment 2-ketogluconate, but retained the ability to ferment gluconate. It was established that 1 mole of carbon dioxide was produced per mole of 2-ketogluconate fermented, although the other products were not identified.

This problem was further investigated to establish the relationship of the 2-ketogluconate fermentation to gluconate fermentation by this organism and to the 2-ketogluconate fermentation by Leuconostoc mesenteroides (Blakley and Blackwood, 1957). Gluconate fermentation by S. faecalis occurs by a mixed pathway, where half of the substrate is oxidized to carbon dioxide and pentose phosphate and the other half is converted to pyruvate and triose phosphate by way of 2-keto-3-deoxy-6-phosphogluconate. 2-Ketogluconate fermentation by L. mesenteroides probably occurs by decarboxylation to pentose phosphate and carbon dioxide, followed by phosphoketolase cleavage of pentose phosphate to acetyl phosphate and triose phosphate.

This report describes studies of growth yields, fermentation balances, and product labeling obtained during growth on, or resting cell fermentation of, 2-ketogluconate by S. faecalis.

MATERIALS AND METHODS

Microbiological methods. The organism used in these studies was S. faecalis 10CI, which was maintained and grown in mass culture as previously described (Sokatch and Gunsalus, 1957). Because no growth was obtained when 2-ketogluconate alone was used, a combination of 30 g of sodium gluconate and 10 g of potassium 2-ketogluconate was used per 15 liters of AC medium (Wood and Gunsalus, 1942). Yield of cell dry weight per mmole of 2-ketogluconate was also done according to published methods (Sokatch and Gunsalus, 1957), with the use of the basal medium of O’Kane and Gunsalus (1948). When carbon dioxide was trapped during growth (Table 1), the growth tube was fitted with a gassing device which allowed the carbon dioxide-free nitrogen to be bubbled through the medium during growth. The gas leaving the growth tube...
was bubbled through standard 0.25 N barium hydroxide which was about 0.085 m in barium chloride (Steele and Sfortunato, 1949). Ordinarily, three such traps were used in series, each containing 3 ml of the barium hydroxide-barium chloride solution. Residual barium hydroxide was titrated with standard 0.1 N hydrochloric acid. Barium carbonate was collected on coarse sintered-glass frits with a Millipore XX10 025 00 filtering assembly. All samples were corrected to infinite thickness, and counts per minute were converted to specific activity and expressed μCi/m mole of carbon.

Fermentation balance studies. Resting-cell fermentations were carried out in a Warburg apparatus under nitrogen. A typical experimental vessel contained 50 μmoles of potassium 2-ketogluconate, 300 μmoles of potassium phosphate buffer (pH 6.0), and cells, equivalent to about 80 mg (dry weight) in a final volume of 3.0 ml. The reaction was followed by measuring carbon dioxide evolution. Large-scale experiments with labeled substrates were done in glass tubes (23 by 200 mm) fitted with a gassing attachment. Nitrogen gas was bubbled through the reaction mixture, and the evolved carbon dioxide was trapped in barium hydroxide-barium chloride, as previously described. A typical reaction mixture in this case would contain 50 μmoles of potassium 2-ketogluconate, 4.0 μmoles of potassium phosphate buffer (pH 6.0), and about 400 mg (dry weight) of cells in a final volume of 24 ml. The fermentation was allowed to proceed for 4.5 hr; the cells were removed by centrifugation in the cold after the addition of 2 ml of 10 N sulfuric acid.

Chemical methods. Glucose-1- and -2-C¹⁴ were purchased from Nuclear-Chicago Corp. (Des Plaines, Ill.). Sodium gluconate was purchased from Pfanzstiehl Chemical Co., Waukegan, III. Lactic acid was determined by the method of Barker and Summerson (1941). Alcohol was separated from the fermentation products by steam distillation after adjusting the pH to 7.0. Alcohol was then determined by oxidation to acetic acid. Samples of 50 ml of the steam distillate were collected in 250-ml flasks with ground-glass joints; 34 ml of the oxidizing agent were added (34 g of Na₄C₂O₄; 2H₂O, 169 ml of 10 N H₂SO₄, and water to make 250 ml). A ground-glass stopper was then wired in place, and the flask was immersed in a boiling water bath for 30 min. The resulting acetic acid was then steam-distilled and titrated with standard base.

Degradation of labeled compounds. Total oxidations were done with the persulfate method as previously described (Sokatch and Günsalus, 1957). Alcohol was separated from the fermentation mixture, as described above, and oxidized to acetic acid for degradation. Acetic acid was degraded according to the method of Phares (1951), except that carbon dioxide was absorbed directly in the barium hydroxide-barium chloride mixture. After the removal of alcohol, the volatile acids, acetic and formic, were removed by steam distillation and titrated with standard base. The steam distillate containing the acid salts was concentrated in an oven at 100 C. Formic acid was oxidized selectively according to the mercuric oxide procedure of Osburn, Wood, and Werkman (1933). This method does not produce oxidation of either acetic or lactic acids. Carbon dioxide from formic acid oxidation was absorbed directly in the barium hydroxide mixture, as already described. Acetic acid was recovered from the mercuric oxide oxidation procedure by steam distillation. The steam-distillate fractions were titrated with standard sodium hydroxide, combined, and taken to dryness in an oven at 100 C. The dried sodium salt was used in the Phares (1951) modification of the Schmidt reaction as described for alcohol. Lactic acid was degraded in the experiments in which arsenite was used. Lactic acid was extracted from the fermentation mixture by continuous ether extraction for 48 hours.

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**Table 1.** C¹⁴O₂ production from 2-ketogluconate by *Streptococcus faecalis* in the presence of other energy sources

<table>
<thead>
<tr>
<th>Energy source</th>
<th>CO₂ evolved μmoles</th>
<th>C¹⁴ content μCi</th>
<th>Per cent of C¹⁴ input</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>59</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glucose</td>
<td>74</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gluconate</td>
<td>110</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2-Ketogluconate-1-C¹⁴</td>
<td>53</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Glucose + 2-ketogluconate-1-C¹⁴</td>
<td>58</td>
<td>0.09</td>
<td>0.72</td>
</tr>
<tr>
<td>Gluconate + 2-ketogluconate-1-C¹⁴</td>
<td>181</td>
<td>7.7</td>
<td>62</td>
</tr>
</tbody>
</table>

* To 10 ml of the basal medium were added 100 μmoles of each substrate. Input of C¹⁴ was 12.4 μCi of 2-ketogluconate-1-C¹⁴.
hr. To separate lactic acid, the extract was chromatographed on silicic acid (Steele, White, and Pierce, 1954), and lactic acid was degraded as previously described (Sokatch, 1960).

**Preparation of potassium 2-ketogluconate.** Because rather large amounts of 2-ketogluconate were required to grow the organism in mass culture, the method of Stubbs et al. (1940) was modified to produce laboratory-scale amounts of 2-ketogluconate. The organism used was a non-pigmented strain of *Pseudomonas aeruginosa* used in other studies in this laboratory (Norton, Bulmer, and Sokatch, 1963). The inoculum was prepared by growing the organism in a medium which contained 12 g of glucose and 0.5 g of yeast extract in 100 ml of water. After this medium had been autoclaved, 2.7 g of calcium carbonate, which had been sterilized separately, were added. The medium was inoculated and incubated overnight at 37 C. The entire contents of the inoculum flask were added to the production flask. The medium for the production of 2-ketogluconate contained 50 g of glucose, 2.5 g of yeast extract, 125 mg of MgSO₄·7H₂O, 300 mg of KH₂PO₄, and 450 ml of water.

A solution of 1 g of urea in 50 ml of water and 13.5 g of calcium carbonate, both of which had been autoclaved separately, was added to the production flask at the time of inoculation. Incubation was at 37 C, with aeration and stirring. When the labeled substrates were prepared, 50 µe of glucose-2H¹⁴ were added the day after the production flask was inoculated. The medium was tested daily for the disappearance of glucose and the appearance of 2-ketogluconate by chromatographing a sample of the flask contents on short strips of Whatman no. 1 paper in tubes (25 by 200 mm) with methyl alcohol, ethyl alcohol, and water, 9:9:2 (Stokes and Campbell, 1951). The papers were sprayed with o-phenylenediamine (De Ley, 1954). When the glucose had disappeared completely, generally after about 7 to 8 days, the cells and calcium carbonate were removed by centrifugation at 12,100 × g in a Servall RC-2 centrifuge for 10 min.

The clarified medium was concentrated with the aid of a rotary evaporator to about 100 ml and was then treated with Dowex 50 (acid form) to remove cations. The treatment with Dowex 50 was repeated until there was no further decrease in pH. The solution was then brought to pH 6.5 with solid potassium hydroxide, although the final adjustment was made with 1 N potassium hydroxide to prevent over-alkalinization. The solution was again reduced in volume, this time to about 30 to 40 ml. Crystalline potassium 2-ketogluconate appeared when the volume was reduced to about 50 ml. This solution was allowed to cool, and the crystalline potassium 2-ketogluconate was harvested by filtration. The filter cake was washed several times with small portions of cold 70% alcohol until the washings were colorless. A second crop of crystals was obtained from the filtrate and washings by again reducing the volume. The yield was 30 to 33 g of potassium 2-ketogluconate.

Elemental analysis of a typical sample of potassium 2-ketogluconate for K, C, and H yielded the following results: K₂C₆H₇O₇(232.168); calculated: K, 16.50; C, 31.00; H, 3.89. Obtained: K, 16.70; C, 31.36; H, 4.04. These results agree closely with the theoretical percentages for K, C, and H. The phenylhydrazide hydrazone was prepared according to the method of Ohle and Berend (1927) and melted at 109 to 110 C, the reported melting point being 108 C. The analytical data fit either potassium 2-ketogluconate or 5-ketogluconate. The latter possibility was ruled out when the periodic acid oxidation technique of Juni and Heym (1962) was used. In this procedure, compounds such as gluconate and 5-ketogluconate, which contain a hydroxyl group at carbon 2, yield glyoxylic acid from carbons 1 and 2. On the other hand, 2-ketogluconate yields glyoxylic acid only after reduction to gluconate with borohydride. This latter result was obtained, thus establishing the identity of our material as potassium 2-ketogluconate. Additionally, it was possible to establish the limit of contamination of 2-ketogluconate with gluconate as about 3%.

**Results**

**Growth of S. faecalis on glucose, gluconate, and 2-ketogluconate.** Preliminary growth experiments with *S. faecalis* in basal medium with 2-ketogluconate as the energy source revealed that no growth occurred at the expense of this substrate. Considering the known pathway of 2-ketogluconate metabolism in *Aerobacter cloacae* (De Ley and Verhoffstede, 1955) and *P. fluorescens* (Frampton and Wood, 1961), which involves reduction of 2-ketogluconate-6-phosphate to 6-phosphogluconate, the possibility was considered that a
catalytic amount of a reducing agent was required to initiate the fermentation. Growth of *S. faecalis* in the presence of gluconate and 2-ketogluconate resulted in an increased yield of cells, as compared with that obtained with gluconate alone. However, when glucose and 2-ketogluconate were used, no greater cell yield was obtained than when glucose alone was used. The lack of stimulation of growth on 2-ketogluconate by glucose was further investigated. Because it was known that resting-cell fermentation of 2-ketogluconate by *S. faecalis* produces carbon dioxide, growth of the organism in the presence of 2-ketogluconate-1-C14 and either glucose or gluconate and measurement of the C14 content of the evolved carbon dioxide was used as an index of 2-ketogluconate fermentation (Table 1). In the case of 2-ketogluconate-1-C14 alone, or in the presence of glucose, no C14 was recovered in the evolved carbon dioxide. In the case of 2-ketogluconate-1-C14 plus gluconate, 62% of the C14 was recovered in the evolved carbon dioxide, and the yield of carbon dioxide was also increased. It thus appears probable that glucose represses the utilization of 2-ketogluconate.

Growth of *S. faecalis* in graded amounts of gluconate with 5 μmoles/ml of 2-ketogluconate resulted in more growth than would be expected from gluconate alone and with a slope about twice that obtained with gluconate alone (Fig. 1). Similarly when the gluconate concentration was kept constant at 5 μmoles per ml and 2-ketogluconate was varied, the increased amount of growth was found to be proportional to the concentration of 2-ketogluconate. The slope of this line was found to be 20 μg (dry weight) per μmole of 2-ketogluconate. These and the preceding data suggest a relationship between the two sugar acids such that 1 mole of 2-ketogluconate is fermented to produce energy for each mole of gluconate present. Hence, the role of gluconate in the fermentation of 2-ketogluconate is not catalytic but stoichiometric. It should be pointed out that these results concern energy production as measured by growth yields, and that resting cells ferment 2-ketogluconate without added gluconate.

**End products of 2-ketogluconate fermentation.**

The results of the studies with resting cells on the fermentation of 2-ketogluconate are given in Table 2. Inspection of these data reveals that 1 mole of carbon dioxide was produced per mole of 2-ketogluconate fermented. The remainder of the carbon was found principally as the products of the elatic reaction, formate, ethanol, and acetate, with surprisingly little lactic acid.

The carbon recovery is somewhat low, approximately 86%, and summation of the oxidized and reduced products reveals that the balance is short in reduced products. This could be explained by a failure to recover all the ethanol produced in the fermentation. The balance is short in C2 products because ethanol plus acetic acid totals 1.1 moles per mole of 2-ketogluconate, whereas 1.42 moles of formate (C3) per mole of 2-ketogluconate were obtained. If the value for ethanol

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**Figure 1. Growth yields of Streptococcus faecalis on gluconate and 2-ketogluconate.**

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**Table 2. End products of 2-ketogluconate fermentation.**

<table>
<thead>
<tr>
<th>Products</th>
<th>μmoles</th>
<th>μmoles of carbon</th>
<th>moles per mole of 2-ketogluconate</th>
<th>O/R value of products*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td>49</td>
<td>49</td>
<td>0.98</td>
<td>+1.96</td>
</tr>
<tr>
<td>Formic acid</td>
<td>71</td>
<td>71</td>
<td>1.42</td>
<td>+1.42</td>
</tr>
<tr>
<td>Ethanol</td>
<td>21</td>
<td>42</td>
<td>0.42</td>
<td>−0.82</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>35</td>
<td>70</td>
<td>0.70</td>
<td>—</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>9</td>
<td>27</td>
<td>0.19</td>
<td>—</td>
</tr>
</tbody>
</table>

*Fermentation balance studies were performed with 50 μmoles of 2-ketogluconate, except for lactic acid determination which was done on a separate 10-μmole fermentation.

† Carbon recovery: 259/300 = 86%.

‡ Total: oxidized, +3.38; reduced, −0.82. Sum of oxidized and reduced products = +2.56; theoretical for 2-ketogluconate, +2.00.
is adjusted accordingly, the fermentation balance for 2-ketogluconate may be represented ideally as: (1) 2-ketogluconate = 1.0 carbon dioxide + 0.2 lactic acid + 1.47 formic acid + 0.73 ethanol + 0.73 acetic acid.

In the study of the gluconate fermentation, it was observed that the addition of $10^{-3}$ M arsenite altered the fermentation, so that the proportion of elastic products was very small (Sokatch and Gunsalus, 1957). The mechanism by which this occurs is not clear because lipoic acid was not implicated in this reaction. The same effect was observed in the case of the 2-ketogluconate fermentation (Table 3). Carbon dioxide production was unaffected, but the yield of lactic acid was raised from 0.19 moles per mole of 2-ketogluconate to 1.33 moles per mole of 2-ketogluconate.

![Image of a chemical structure](http://jb.asm.org/)

**FIG. 2.** Formation of labeled hexose and triose phosphates from 2-ketogluconate-2-C$^{14}$. 5 moles of 2-ketogluconate-2-C$^{14}$ are converted to 3 moles each of carbon dioxide and pentose phosphate-1-C$^{14}$. The latter are converted to hexose and triose phosphate, with the indicated labeling pattern, by means of the sedoheptulose diphosphate pathway found in this organism (Sokatch, 1960, 1962). The usual sequence of transketolase and transaldolase would also provide the same labeling pattern. Hexose and triose phosphates are then converted to pyruvate-1,3-C$^{14}$ by the usual Embden-Meyerhof pathway enzymes.

**TABLE 3.** Effect of $10^{-3}$ M arsenite on gluconate and 2-ketogluconate fermentation

<table>
<thead>
<tr>
<th>Additions</th>
<th>Carbon dioxide (μmoles)</th>
<th>Lactic acid (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Arsenite</td>
<td>+ Arsenite</td>
</tr>
<tr>
<td>None</td>
<td>0.89</td>
<td>1.2</td>
</tr>
<tr>
<td>Gluconate, 10 μmoles</td>
<td>6.1</td>
<td>5.9</td>
</tr>
<tr>
<td>2-Ketogluconate, 10 μmoles</td>
<td>10.4</td>
<td>10.4</td>
</tr>
</tbody>
</table>

**TABLE 4.** Product labeling in 2-ketogluconate-1-C$^{14}$ and -2-C$^{14}$ fermentation

<table>
<thead>
<tr>
<th>Product</th>
<th>2-Ketogluconate-1-C$^{14}$</th>
<th>2-Ketogluconate-2-C$^{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>Isotope enrichment</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>120</td>
<td>5.8</td>
</tr>
<tr>
<td>Formic acid</td>
<td>15</td>
<td>0.7</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>-CH$_2$OH</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>-CH$_3$</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td>-COOH</td>
<td>0.2</td>
<td>0.01</td>
</tr>
</tbody>
</table>


† Expressed as specific activity of product/ specific activity of 2-ketogluconate.
TABLE 5. Product labeling in 2-ketogluconate-1- and
2-C¹⁴ fermentation in the presence of
10⁻³ M arsenite

<table>
<thead>
<tr>
<th>Product</th>
<th>2-Ketogluconate-1-C¹⁴</th>
<th>2-Ketogluconate-2-C¹⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>Isotope enrichment</td>
</tr>
<tr>
<td>Carbon acid</td>
<td>107</td>
<td>5.2</td>
</tr>
<tr>
<td>Lactic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-COOH</td>
<td>12</td>
<td>0.58</td>
</tr>
<tr>
<td>-CHOH</td>
<td>0†</td>
<td>—</td>
</tr>
<tr>
<td>-CH₄</td>
<td>0†</td>
<td>—</td>
</tr>
</tbody>
</table>

*All conditions identical to those in Table 4 with the exception that fermentations were done in the presence of 10⁻³ M arsenite.
† Determined by persulfate oxidation of acetic acid from the permanganate oxidation of lactic acid.
‡ Determined by Schmidt degradation of acetic acid from the permanganate oxidation of lactic acid.

would then arise from these compounds by means of the usual Embden-Meyerhof reactions.

The same pathway with 2-ketogluconate-1-C¹⁴ would result in the bulk of the isotope being found in carbon dioxide. Explanation of the significant amount of label found in formate in this case is most readily accomplished by assuming that a portion of the 2-ketogluconate is routed through the Entner-Doudoroff pathway (Entner and Doudoroff, 1952) which would produce pyruvate labeled in the carboxyl group.

These same fermentation studies were repeated in the presence of 10⁻³ M arsenite; in this case, the isotope distribution of lactic acid was studied (Table 5). In the case of 2-ketogluconate-1-C¹⁴, again the isotope was found largely in carbon dioxide. About 10% was recovered in the carboxyl group of lactic acid corresponding to that amount recovered in formate when arsenite was not used. In the case of 2-ketogluconate-2-C¹⁴, the methyl and carboxyl groups of lactic acid were labeled in the proportion of 2:1 as expected. It is clear that arsenite did not change the major course of the fermentation, but rather changed the distribution of end products formed from pyruvate.

DISCUSSION

The data from the experiments with 2-ketogluconate-1- and -2-C¹⁴ are best interpreted as a decarboxylation of 2-ketogluconate to carbon dioxide and pentose phosphate. This could occur by phosphorylation of 2-ketogluconate, reduction to 6-phosphogluconate, and oxidation to ribulose 5-phosphate and carbon dioxide. As was found in the case of the gluconate fermentation in this same organism, pentose phosphate would then be converted to hexose phosphate and fermented by way of the Embden-Meyerhof pathway. In *S. faecalis*, transaldolase appears to be lacking (Sokatch, 1960); however, its function may be served by a combination of phosphofructokinase and aldolase. Sedoheptulose 1,7-diphosphate is an intermediate and is converted to fructose 1,6-diphosphate by aldolase. The labeling pattern and energy yield are identical with that obtained with the use of transaldolase (Sokatch, 1962).

(a) 3 2-ketogluconate + 3 adenosine triphosphate (ATP) → 3 carbon dioxide + 3 pentose phosphate

(b) 2 pentose phosphate → sedoheptulose 7-phosphate + triose phosphate

(c) sedoheptulose 7-phosphate + ATP → sedoheptulose 1,7-diphosphate + adenosine diphosphate

(d) sedoheptulose 1,7-diphosphate + triose phosphate → fructose 1,6-diphosphate + erythrose 4-phosphate

(e) erythrose 4-phosphate + pentose phosphate → fructose 6-phosphate + triose phosphate

Sum: 3 2-ketogluconate + 3 ATP = fructose 1,6-diphosphate + fructose 6-phosphate + triose phosphate

By this scheme, the isotope from 2-ketogluconate-1-C¹⁴ would be recovered in the carbon dioxide at a specific activity six times that obtained in the total combustion of 2-ketogluconate-1-C¹⁴. With similar reasoning, fermentation of 2-ketogluconate-2-C¹⁴ should result in pyruvate labeled in the methyl and carboxyl carbons at a specific activity 2.4 and 1.2 times, respectively, that obtained in the total oxidation of 2-ketogluconate (see also Fig. 2). The data presented in Tables 4 and 5 are generally in agreement with these expectations, assuming the usual reactions leading from pyruvate to the obtained end products.

It is possible to calculate an energy yield per mole of 2-ketogluconate fermented on the basis of the above interpretation of the labeling data and the balance presented in equation 1. The pathway from pentose to hexose phosphate...
through sedoheptulose diphosphate yields 1.67 moles of ATP per mole of pentose, as does the transaldolase pathway (Elsden and Peel, 1955). Assuming that 90% of the 2-ketogluconate is fermented by this route, the energy yield would be 0.9 × 1.67 = 1.51 moles of ATP per mole of 2-ketogluconate. By a similar process, the ATP contribution from the Entner-Doudoroff pathway, which yields a net of 1 mole of ATP per mole of hexose, would in this case be 0.1 mole of ATP per mole of 2-ketogluconate. Finally, 1 mole of ATP is available from each mole of acetate through the action of the enzyme acetokinase. Based on the theoretical balance presented in equation 1, 0.73 moles of ATP per mole of 2-ketogluconate would be produced. The total calculated ATP yield would then be 2.34 moles per mole of 2-ketogluconate. The slope of the curve in Fig. 1, obtained by subtracting the growth due to gluconate from that obtained in the presence of both sugar acids is 19 µg (dry weight) per µmole of 2-ketogluconate. Assuming a dry-weight yield of about 10 µg/µmole of ATP (Bauchope and Elsdon, 1960), then it may be estimated from this method that 1.9 µmole net of ATP are produced per µmole of 2-ketogluconate, which is within reasonable range of the calculations. The ATP yield in the gluconate fermentation may also be corrected for the acetate produced, in this case about 0.5 µmole of acetate per mole of gluconate. The final ATP yield from gluconate then would be about 1.8 µmole per µmole of gluconate fermented. The slope of the curve for growth on gluconate alone (Fig. 1) is 20 µg (dry weight) per µmole of gluconate.

The failure of S. faecalis to grow on 2-keto-gluconate alone is not understood. The ability of the organism to ferment 2-ketogluconate in the resting-cell experiments without added gluconate indicates that a complete pathway exists for the metabolism of 2-ketogluconate in the cells used. On the other hand, the apparent stoichiometric requirement for gluconate during growth suggests that gluconate is somehow involved in energy metabolism of 2-ketogluconate, possibly in the early stages of metabolism. This may cause some revision in the preceding calculations of energy yield.

The conversion of a rather large proportion of 2-ketogluconate to ethanol, acetic acid, and formic acid recalls the fermentation of galactose by S. pyogenes (Steele et al., 1954) and S. faecalis (Fukuyama and O’Kane, 1962). This phenomenon was explained by Eisenberg and O’Kane (1963) as a result of a competition for nicotinamide adenine dinucleotide (NAD) by lactic dehydrogenase and uridine diphosphate galactose 4-epimerase, with the result that the NAD-nonrequiring lactic system is favored in the competition for pyruvate. A similar situation can be visualized in the case of the 2-ketoglucanate fermentation, because there is reason to believe that the initial steps in the fermentation involve reduction of the substrate, probably with a pyridine nucleotide co-factor.

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Literature Cited


