Lactate Metabolism by *Veillonella parvula*

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A strain of *Veillonella parvula* M4, which grows readily in lactate broth without a requirement for carbon dioxide, has been isolated from the oral cavity. Anaerobic, washed cells of this organism fermented sodium lactate to the following products (moles/100 moles of lactate): propionate, 66; acetate, 40; carbon dioxide, 40; and hydrogen, 14. Cells grew readily in tryptone-yeast extract broth with pyruvate, oxaloacetate, malate, and fumarate, but poorly with succinate. The fermentation of pyruvate, oxaloacetate, or lactate plus oxaloacetate by washed cells resulted in the formation of propionate and acetate in ratios significantly lower than those observed with lactate as the sole carbon source. This was primarily due to increased acetate production. Cell-free extracts were unable to degrade lactate but metabolized lactate in the presence of oxaloacetate, indicating the presence of malic-lactic transhydrogenase in this organism. Lactic dehydrogenase activity was not observed. Evidence is presented for oxaloacetate decarboxylase and malic dehydrogenase activities in extracts.

Members of the genus *Veillonella* are unable to utilize carbohydrates but readily metabolize purines and short-chain acids, such as lactate and pyruvate, for energy (6, 11). An extensive review of the characteristics and properties of the *Veillonella* (16, 18, 19) prompted Rogosa (17) to revise the classification of these obligate anaerobes into two main species: *V. alcalescens* and *V. parvula*.

Foubert and Douglas (6) first reported in detail on the metabolism of members of the *Veillonella* by showing that *Micrococcus lactilyticus*, now classified as *V. alcalescens* (17), fermented lactic acid with the production of hydrogen, carbon dioxide, acetate, and propionate. In the same year, Johns (10) isolated a "new micrococcus" from the rumen of sheep which was unable to ferment carbohydrates but was capable of degrading pyruvate, lactate, oxaloacetate, malate, and fumarate to the same end products as those of *M. lactilyticus*. Succinate fermentation, on the other hand, resulted in the formation of only propionate and carbon dioxide. Johns (10, 11) identified the "new micrococcus" as *V. gazogenes* (*V. alcalescens*) and showed that the utilization of lactate by this strain had a mandatory requirement for carbon dioxide. Rogosa (16) confirmed this by demonstrating that strains of both type species fermented lactate poorly, or not at all, in the absence of carbon dioxide. Furthermore, even in the presence of carbon dioxide, lactate metabolism by these strains was limited.

The long and confused history of *Veillonella* taxonomy has made it difficult to make comparisons between the metabolism of *V. parvula* and that of *V. alcalescens*. As pointed out by Rogosa (16), most investigators have generally designated any veillonella isolate as *V. alcalescens*; consequently, the literature contains few reports of studies with *V. parvula*. With this in mind, a study was undertaken to examine the metabolic properties of *V. parvula* species isolated from the human oral cavity. During the course of this study, a strain of *V. parvula* (M4) was obtained which differed from the strains of *Veillonella* reported previously, in that it grew and metabolized sodium lactate readily in the absence of carbon dioxide. This report describes the ability of this organism to degrade lactate and other short-chain acids with the formation of propionate, acetate, carbon dioxide, and hydrogen. Data will be presented elsewhere demonstrating the involvement of carbon dioxide fixation in the formation of propionate by this strain (I. R. Hamilton and K. C. S. Ng, manuscript in preparation).

**MATERIALS AND METHODS**

Strains of *V. parvula* were isolated anaerobically and with the aid of antibiotics (15, 20) from human saliva by employing Rogosa's 1% lactate medium (16) supplemented with phosphate buffer (0.1 M, pH 6.5). The viability and purity of the cultures was checked at monthly intervals by the staining procedures and routine taxonomic tests described by Rogosa (16) and Rogosa and Bishop (18, 19). The *Veillonella* species isolated were classified by the scheme proposed by Rogosa (17).

The strain selected for study, *V. parvula* M4, had the following characteristics: gram-negative; obligate anaerobe; small coccolid (0.4 μm in diameter); unable to
ferment carbohydrates; grew in tryptone-yeast extract broth with lactate (growth-rate constant = 0.49), pyruvate (0.49), oxaloacetate (0.25), malate (0.26), fumarate (0.25), and succinate (0.19); produced H₂S; produced nitrite from nitrate; was indole-, gelatin-, starch- and catalase-negative; and was beta-hemolytic on blood agar. Putrescine and cadaverine were not required for growth in semidefined basal medium containing 1% lactate. The basal medium employed was that proposed by Rogosa and Bishop (18) without hypoxanthine, uracil, and putrescine.

**Preparation of resting cells and cell-free extracts.** Large quantities of exponential-phase cells were routinely obtained from heavily inoculated anaerobic lactate broth cultures of *V. parvula M4* grown in 1- to 2-liter flasks. Growth was followed turbidimetrically in a Klett-Summerson colorimeter containing a red filter (640 to 700 nm). Exponential-phase cells were collected by centrifugation at 13,000 × g for 10 to 15 min in a LRA Lourdes refrigerated centrifuge at 4 C. The cells were washed once and ultimately suspended in degassed phosphate buffer (0.1 m, pH 6.5) containing 20 mM MgSO₄ and 20 mM 2-mercaptoethanol; these conditions were optimal for lactate fermentation with resting cells. Reaction mixtures routinely contained 2 mg (dry weight) of cells per ml.

Cell-free extracts were obtained from exponential-phase cells (30 mg [dry weight]/ml) either by sonic disruption for 15 min in a stream of nitrogen in a Raytheon sonic oscillator at 4 C or by passing washed cells through a French pressure cell (American Instruments Co.) three to four times at a pressure of 6,000 to 9,000 psi. The broken-cell preparations were centrifuged at 13,000 × g for 15 min at 4 C, and the dark, reddish brown supernatant was stored at 0 C under nitrogen until used.

All manipulations involving cells or extract preparations were carried out in a stream of nitrogen to maintain anaerobic conditions.

**Experimental procedure.** Experiments were normally carried out at 37 C in 25- to 50-ml Erlenmeyer flasks fitted with tight fitting serum stoppers except when gas production was determined. The flasks containing washed cells or extract preparations in buffer were evacuated and gassed with nitrogen via a syringe needle three to four times, and the substrate was added by syringe to start the reaction. To stop the reaction, samples were withdrawn at various times by syringe and added either to 2 ml of 0.05 N NaOH or to 0.1 ml of 25% ZnSO₄ in plastic centrifuge tubes. After neutralization, the supernatant fluid obtained by centrifugation was recovered and frozen until analyzed. When keto acids were to be measured, samples were added to an equal volume of 0.1% 2,4-dinitrophenylhydrazine in 2 N HCl, and the mixture was incubated at 4 C for 60 min. Some of the hydrazones formed precipitated during this procedure and were separated from cells or protein by extraction with ethyl acetate.

One unit of metabolic activity is defined as the degradation of 1 μmole of substrate per mg (dry weight) of cell material (or mg of protein) per hour.

**Analyses.** Lactic acid was assayed by the modified lactic dehydrogenase method of Cohen and Noell (3). Pyruvic acid was assayed either colorimetrically with 2,4-dinitrophenylhydrazine (9) or enzymatically with lactic dehydrogenase (8). Oxaloacetate was measured either colorimetrically with 2,4-dinitrophenylhydrazine, enzymatically with malic dehydrogenase, or by the direct spectrophotometric method of Allen (1). The enzymatic assay contained 0.2 ml of sample (0 to 10 μg of oxaloacetate) and 0.8 ml of buffer-reduced nicotinamide adenine dinucleotide (NADH)-enzyme solution consisting of 90 nmoles of NADH and 0.05 μg of malic dehydrogenase (Boehringer Mannheim Corp.) in either phosphate or tris (hydroxymethyl)aminomethane buffer (0.05 m, pH 7.5). The remaining NADH was measured spectrophotometrically at 340 nm after incubation for 20 min at room temperature. Freshly prepared sodium oxaloacetate solutions were used as standards. The presence of malic dehydrogenase activity in *V. parvula* was determined in the same assay system by substituting cell-free extract, prepared by sonic treatment, for the commercial malic dehydrogenase. Protein was determined by the ultraviolet-adsorption method of Layne (13).

**Gasous products.** The extreme oxygen sensitivity of *V. parvula M4* militated against the use of conventional manometric methods for the measurement of carbon dioxide and hydrogen evolution. For this purpose, a modification of the triple side-arm Warburg method of Hamilton et al. (9) was employed, which gave consistent results for measurement of carbon dioxide and hydrogen in the same flask. The modification consisted of adding the cells or extract preparation to the flask after the nitrogen-gassing procedure by syringe via the normal side-arm which had been stopped with a tight fitting serum cap.

**Nongaseous products.** Nongaseous end products formed during metabolism were separated by silicic acid column chromatography employing a 0 to 15% butanol-chloroform gradient (2). All solvents were saturated with 0.5 N H₂SO₄. Acidified 15% butanol in chloroform was added by peristaltic pump at a rate of 0.5 ml/min to 100 ml of acidified chloroform in a 250-ml mixing chamber. The contents of this chamber were then added to a column (1.3 by 20 cm) of 100- to 200-mesh silicic acid which had been equilibrated with 0.5 N H₂SO₄. The preparation of the column and sample has been previously described (9). Fractions (3 ml) were collected, and the acidity was determined in duplicate 1-ml samples by titrating to the phenolphthalein end point with standardized NaOH. The radioactivity content of the remaining portion was determined by liquid scintillation counting.

**Preparation of lactate-U-¹⁴C.** Uniformly labeled "C-lactate was prepared enzymatically from sodium pyruvate-U-¹⁴C by the method of Krebs et al. (12) or isolated from the medium after the anaerobic metabolism of glucose-U-¹⁴C by the homofermentative organism *Streptococcus salivarius* under conditions previously described (8).

**RESULTS**

**Growth.** The growth-rate constant for *V. parvula M4* in lactate broth was 0.49, calculated from an average generation time of 88 min, and was the same whether nitrogen or nitrogen plus
parvula M4 in parvula dium. The yield of cell material, however, was slightly higher when carbon dioxide was present. Furthermore, carbon dioxide had a similar effect on V. parvula M4 growing anaerobically in defined medium with 1% lactate. The growth rate was constant between 30 and 37 C, with marked inhibition at 40 C and above. However, cultures were routinely grown at 31 C as large amounts of viscus extracellular material were produced at 37 C, hindering centrifugation of the cells. The pH of the medium remained constant throughout the growth cycle.

Lactate metabolism by resting cells. The rate of lactate metabolism by resting cells incubated under optimal conditions in phosphate buffer varied with the substrate concentration in a typical enzyme-substrate manner (Fig. 1). From 0 to 15 mM sodium lactate, the rate of utilization was first order, whereas at levels between 15 and 80 mM, the rate was zero order with a V_max of 102 units, i.e., 102 μmoles of lactate utilized per mg (dry weight) of cells per hr. As severe and progressive substrate inhibition was observed above 80 mM, the initial substrate concentration in all experiments was kept between 15 and 80 mM. Like growing cultures, resting cell metabolism did not result in a change in the pH of the medium.

V. parvula M4, like V. alcalenscens (6, 10), degraded sodium lactate anaerobically to acetate, and propionate, carbon dioxide, and hydrogen. The time-course of product formation (Fig. 2) shows that carbon dioxide and propionate were produced in greater abundance as compared to hydrogen and acetate acid, respectively. In all cases, product formation from stored energy material was negligible. The small incorporation of radioactivity into cells during the course of lactate-U-14C fermentation under the same conditions confirms this conclusion.

Table 2 shows the fermentation balance for V. parvula M4 in comparison to that obtained by Foubert and Douglas with M. lactilyticus (6) and by Johns with V. gazogenes (10). The results with V. parvula M4 are similar to those obtained by Foubert and Douglas with M. lactilyticus. Endogenous metabolism contributed about 6% to

<table>
<thead>
<tr>
<th>Gas phase</th>
<th>Growth rate constant</th>
<th>Yield*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N_2</td>
<td>0.49</td>
<td>0.043</td>
</tr>
<tr>
<td>N_2 + 5% CO_2</td>
<td>0.49</td>
<td>0.053</td>
</tr>
</tbody>
</table>

* Cell dry weight (gram) per gram of sodium lactate.

FIG. 1. Effect of substrate concentration on the rate of lactate utilization by washed cells of V. parvula M4. Resting cells of V. parvula M4 were incubated at 2 mg (dry wt)/ml with various concentrations of sodium lactate at 37°C in an atmosphere of N_2. Samples were removed periodically, the remaining lactate was assayed, and the initial rate (micromoles per milligram of dry cells per hour) was determined.

FIG. 2. Products of lactate metabolism by washed, resting cells of V. parvula M4 incubated in a nitrogen atmosphere. (A) Carbon dioxide, O; hydrogen, □; sodium lactate, ■. (B) Acetate, Δ; propionate, Δ.
the end products as observed from the carbon recovery, i.e., 105.5%.

Related metabolism. Johns (11) proposed a pathway for the degradation of lactate by *V. gazzogenes* which was similar to that proposed by Delwiche (4) for *Propionibacterium pentosaceum*. In this pathway, lactate was thought to be degraded initially to pyruvate which is then either decarboxylated to the end products acetate, carbon dioxide, and hydrogen or converted to oxaloacetate by a carbon dioxide fixation reaction. Oxaloacetate is then further metabolized to malate, fumarate, and succinate. The final step is the decarboxylation of succinate to form propionate and carbon dioxide.

To test whether this pathway might be operable in *V. parvula* M4, the ability of washed cells to degrade these compounds was determined. Anaerobic cells readily fermented pyruvate and oxaloacetate (Fig. 3A), although the initial rates of degradation (70 and 48 units, respectively) were considerably lower than that for lactate (102 units). Although the degradation of lactate by malic-lactic transhydrogenase in species of *Veillonella* requires oxaloacetate (E. F. Phares and M. V. Long, 1956, Amer. Chem. Soc. Meeting Abstr., p.626), the incubation of cells with both oxaloacetate and lactate resulted in a marked decrease in the rate of utilization of both substrates (Fig. 3B). The initial rates of 66 and 26 units for lactate and oxaloacetate, respectively, were almost half of the rates obtained when the cells were incubated with each substrate alone, indicating that they possibly compete for enzymes in the same pathway.

Further insight into the reactions involved in lactate metabolism in *V. parvula* was obtained by quantitative determination of the acidic end products formed during the metabolism of pyruvate, oxaloacetate, and lactate-\(^{14}C\) plus oxaloacetate. These substrates, like lactate, were degraded to propionate and acetate (Table 3), as well as to carbon dioxide and hydrogen. However, the propionate to acetate ratios for these substrates were appreciably lower than that obtained with lactate alone. The lowest ratios were obtained when pyruvate or oxaloacetate were the substrates (0.46 and 0.44, respectively) indicating that the metabolism of these substrates was primarily in the direction of acetate formation. The incubation of lactate-\(^{14}C\) with oxaloacetate (Table 3) resulted in decreased acetate formation as compared to acetate production when oxaloacetate was the sole carbon source.

A comparison of the \(^{14}C\)-specific activities of the acetate and propionate formed in experiments 1 and 4 indicates that the addition of 10 mm unlabelled sodium oxaloacetate to 10 mm sodium lactate-\(^{14}C\) resulted in a decrease of only 14% in the propionate specific activity, whereas that of acetate, on the other hand, was reduced 61%. The data in Table 3 indicate that lactate-\(^{14}C\) contributed 96% of the carbon in propionate when it was the sole energy source, with 4% arising from

**TABLE 2. Fermentation balance obtained for *V. parvula* M4 compared to those obtained by Foubert and Douglas (6) and Johns (10) for *V. alcalescens***

<table>
<thead>
<tr>
<th>Product</th>
<th><em>V. parvula M4</em></th>
<th><em>V. alcalescens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium lactate fermented</td>
<td>Foubert-Douglas (6)</td>
<td>Johns (10)</td>
</tr>
<tr>
<td>Propionate</td>
<td>66.0</td>
<td>63.7</td>
</tr>
<tr>
<td>Acetate</td>
<td>39.6</td>
<td>39.5</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>38.3</td>
<td>39.1</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>13.6</td>
<td>14.2</td>
</tr>
<tr>
<td>Carbon recovery</td>
<td>105.5</td>
<td>103.6</td>
</tr>
<tr>
<td>O/R ratio</td>
<td>0.96</td>
<td>0.9</td>
</tr>
</tbody>
</table>

![Fig. 3. Metabolism of \(^{14}C\)-lactate, oxaloacetate, pyruvate, and \(^{14}C\)-lactate plus oxaloacetate by resting cells of *V. parvula* M4 in an atmosphere of nitrogen + 5% carbon dioxide. (A) Individual flasks contained 10 mm of the following: sodium lactate-\(^{14}C\) (1.491 dpm/mole), potassium pyruvate, sodium oxaloacetate. △. (B) Combined incubation of 10 mm sodium lactate-\(^{14}C\) plus 10 mm sodium oxaloacetate: sodium lactate-\(^{14}C\), potassium pyruvate, sodium oxaloacetate. △.](http://jb.asm.org/Downloaded-from)
endogenous material. However, with lactate-\textsuperscript{14}C plus oxaloacetate, 83% of the propionate originated from the lactate and 14% from the unlabeled oxaloacetate. On the other hand, 94% of the acetate carbon originated from lactate-\textsuperscript{14}C when it was the sole energy source but only 37% when oxaloacetate was the additional substrate. In this case, oxaloacetate provided 57% of the acetate carbon, indicating the presence of an active oxaloacetate decarboxylase in cells of \textit{V. parvula} M4. Endogenous metabolism contributed 4 to 6% of the carbon to the respective end products.

**Metabolism of cell-free extracts.** The initial oxidation of lactate by species of \textit{Veillonella} was shown to be catalyzed by malic-lactic transhydrogenase, which couples the oxidation of lactate to pyruvate with the reduction of oxaloacetate to malate (1, 5; E. F. Phares and M. V. Long, 1956, Amer. Chem. Soc. Meeting Abstr., p. 626). The presence of this enzyme in \textit{V. parvula} M4 was indicated by the inability of cell-free extracts to degrade sodium lactate-\textsuperscript{14}C (Fig. 4A). However, when oxaloacetate was incubated with lactate in equimolar quantities, both substrates were degraded at an initial rate of 36 units (Fig. 4B).

No lactic dehydrogenase activity was observed in cell-free extracts of \textit{V. parvula} M4 assayed either with lactate at pH 6.5 and 10.0 or with pyruvate at pH 6.5.

End-product analysis of the 60-min sample from the flask containing lactate-\textsuperscript{14}C plus oxaloacetate and sodium pyruvate is presented in Table 3 and Fig. 4B.

**TABLE 3.** Acidic end products produced by resting cells of \textit{V. parvula} M4 incubated with \textsuperscript{14}C-lactate, pyruvate, oxaloacetate, and \textsuperscript{14}C-lactate plus oxaloacetate in an atmosphere of nitrogen.

<table>
<thead>
<tr>
<th>Expt</th>
<th>Substrate</th>
<th>Propionate</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conc\textsuperscript{a}</td>
<td>Specific activity\textsuperscript{c}</td>
</tr>
<tr>
<td>1</td>
<td>Lactate-\textsuperscript{14}C</td>
<td>60.7</td>
<td>1,428</td>
</tr>
<tr>
<td>2</td>
<td>Pyruvate</td>
<td>34.8</td>
<td>80.0</td>
</tr>
<tr>
<td>3</td>
<td>Oxaloacetate</td>
<td>29.0</td>
<td>52.0</td>
</tr>
<tr>
<td>4</td>
<td>Lactate-\textsuperscript{14}C + oxaloacetate</td>
<td>31.3</td>
<td>1,226</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Moles/100 moles of total substrate.
\textsuperscript{b} Distinctions per minute per micromole.
\textsuperscript{c} Lactate-\textsuperscript{14}C specific activity = 1,491 dpm/\textmu mole.

**TABLE 4.** Distribution of radioactive carbon in propionate and acetate resulting from the utilization of lactate-\textsuperscript{14}C plus oxaloacetate by cell-free extracts of \textit{V. parvula} M4.

<table>
<thead>
<tr>
<th>Products</th>
<th>Conc\textsuperscript{a}</th>
<th>Specific activity\textsuperscript{b}</th>
<th>Per cent contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oxaloacetate + endogenous</td>
</tr>
<tr>
<td>Propionate</td>
<td>34.1</td>
<td>538</td>
<td>30.7 69.3</td>
</tr>
<tr>
<td>Acetate</td>
<td>47.8</td>
<td>776</td>
<td>41.4 58.6</td>
</tr>
<tr>
<td>Propionate/acetate ratio</td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Specific activity = 1791 dpm/\textmu mole.
\textsuperscript{b} Moles/100 moles of total substrate.
\textsuperscript{c} Distinctions per minute per micromole.

**FIG. 4.** Metabolism of pyruvate, oxaloacetate, and lactate plus oxaloacetate by cell-free extracts of \textit{V. parvula} M4 in an atmosphere of nitrogen. (A) Individual flasks contained 5 mg of protein/ml and 10 mm of the following: sodium lactate-\textsuperscript{14}C (1,491 dpm/\textmu mole), ; potassium pyruvate, ; sodium oxaloacetate, . (B) Combined incubation of 10 mm sodium lactate-\textsuperscript{14}C plus 10 mm sodium oxaloacetate: sodium lactate-\textsuperscript{14}C, ; sodium oxaloacetate, .
acetate (Fig. 4B) demonstrated that the degradation of these two substrates resulted in the formation of radioactive propionate and acetate in a ratio of 0.71 (Table 4), which was somewhat higher than that for resting cells (0.60). The distribution of $^{14}$C into the acidic end products showed that the radioactive lactate contributed 30% of the propionate carbon, whereas the remaining 70% originated with oxaloacetate plus endogenous sources. As lactate-$U^{-14}$C was not metabolized by cell-free extracts, it was not possible to calculate the contribution of carbon from endogenous sources. Oxaloacetate plus endogenous material contributed slightly more to acetate production than did the lactate substrate.

Pyruvate and oxaloacetate were also metabolized by extracts, although the rates (13.3 and 19.5 units, respectively) were lower than that for the degradation of lactate plus oxaloacetate. Results to be presented elsewhere (in preparation) demonstrate that pyruvate degradation proceeds principally via the "phosphoroclastic" reaction (14) to acetate, carbon dioxide, and hydrogen with only small quantities of propionate being formed. Acetate and carbon dioxide were also the major products of oxaloacetate metabolism, indicating that this substrate was decarboxylated by oxaloacetate decarboxylase followed by phosphoroclastic cleavage of the pyruvate formed. Furthermore, as malic dehydrogenase activity was observed in extracts of V. parvula M4, it is clear that oxaloacetate can also be converted to malate (Table 5). The enzyme was completely inhibited by 10 mM malonate.

**DISCUSSION**

The stimulation of lactate metabolism in V. gazogenes (11) by carbon dioxide and bicarbonate, as well as in those strains studied by Rogosa (16), is an important characteristic differentiating these organisms from our strain of V. parvula.

**Table 5. Oxidation of reduced nicotinamide adenine dinucleotide (NADH) by cell-free extracts of V. parvula M4 in the presence and absence of oxaloacetate (OAA)**

<table>
<thead>
<tr>
<th>Additions to basic system*</th>
<th>Rate of NADH oxidation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH</td>
<td>612</td>
</tr>
<tr>
<td>NADH + OAA</td>
<td>9,600</td>
</tr>
<tr>
<td>NADH + OAA + malonate</td>
<td>12</td>
</tr>
</tbody>
</table>

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* Basic system: extract (2.5 mg/ml) in 0.05 M phosphate buffer, pH 6.5.
† Rate of NADH oxidation in nanomoles of NADH oxidized per milligram of protein per hour. Oxidation was measured as the loss of NADH at 340 nm.
‡ Component omitted from the blank.

The growth of V. parvula M4 in lactate broth was not carbon dioxide-dependent (Table 1) nor was the rate of lactate metabolism by washed cells altered by the presence or absence of carbon dioxide in the gas phase (in preparation). Furthermore, the rate and extent of lactate fermentation as observed by Johns (11) and Rogosa (16) was still limited, even in the presence of carbon dioxide. The latter author reported that only 6 μmoles of lactate was metabolized by 20 mg (dry weight) of cells in 2 hr. By contrast, Fig. 3 illustrates the degradation of 5 μmoles of lactate per mg (dry weight) of V. parvula M4 cells in 5 min. In addition, higher substrate to cell ratios produced higher rates to a maximum of 102 μmoles of lactate metabolized per mg (dry weight) of cells per hour (Fig. 1).

The stoichiometry of lactate metabolism by V. parvula M4 from the fermentation balance (Table 2) can be depicted in equation 1:

\[
8 \text{lactate} \rightleftharpoons 5 \text{propionate} + 3 \text{acetate} + 3 \text{CO}_2 + \text{H}_2 \quad (1)
\]

This equation is supported by the fermentation data obtained with V. parvula M4 and with M. lactilyticus (6). The ratios of the end products formed by V. gazogenes (11), however, vary slightly because equimolar quantities of acetate and carbon dioxide were not obtained. The pathway of lactate degradation proposed by Johns (11) suggests that every molecule of carbon dioxide fixed in the formation of oxaloacetate is recovered in the subsequent decarboxylation of succinate. Thus, the degradation of pyruvate to acetate, carbon dioxide, and hydrogen would be the only reaction responsible for net carbon dioxide evolution, such that acetate, carbon dioxide, and hydrogen should theoretically appear in equimolar quantities. This is confirmed by more recent evidence that methylmalonyl-coenzyme A is ultimately decarboxylated to propionate and carbon dioxide (7), rather than being coupled to oxaloacetate formation from pyruvate by methylmalonyl-coenzyme A transcarboxylase, as is the case with the propionibacteria (21).

The validity of equation 1 can be tested by examining the hypothetical production and consumption of reducing power at various steps of lactate metabolism by V. parvula M4. By approximating the values in Table 2 for this organism, it can be seen that 106 moles of substrate lactate (100 moles added plus 5.5 moles of endogenous "lactate" as calculated from total recovery) produced the following moles of product: propionate, 66; acetate, 40; carbon dioxide, 40; hydrogen, 14. Accordingly, the initial conversion of lactate by malic-lactic transhydrogenase (equation 2) would require the presence of 106 moles of oxaloacetate and would produce 106...
moles of pyruvate and malate.

\[ \text{lactate} + \text{oxaloacetate} \rightarrow \text{pyruvate} + \text{malate} \] (2)

A reaction producing reducing power during lactate metabolism is the phosphorolactic degradation of pyruvate. From the fermentation balance (Table 2), 40 moles of pyruvate was theoretically degraded to 40 moles of acetate, carbon dioxide, and hydrogen. However, only 14 moles of hydrogen was actually produced by hydrogenase during metabolism, indicating that 26 electron pairs (40 – 14 = 26) were utilized to reduce another component of the system.

The remaining pyruvate (106 – 40 = 66) would be converted to 66 moles of oxaloacetate by carbon dioxide fixation, and the oxaloacetate thus formed would be subsequently degraded to 66 moles of propionate. Net carbon dioxide consumption is not involved in this pathway as succinate decarboxylation regenerates any carbon dioxide fixed with pyruvate. Obviously, the oxaloacetate formed during this reaction could drive malic-lactic transhydrogenase with the net formation of 66 moles of malate and pyruvate. However, as 106 moles of lactate was consumed, an additional 40 moles of oxaloacetate would have to be generated for this purpose. This could be accomplished by recycling the malate formed in the transhydrogenase reaction back to oxaloacetate either by the combined action of malic enzyme and pyruvate carboxylase (in preparation) or directly with malic dehydrogenase without disturbing the stoichiometry of propionate production, i.e., 66 moles of oxaloacetate to 66 propionate. The net effect would be the production of reducing power in the form of 40 moles of NADH or reduced nicotinamide adenine dinucleotide phosphate (NADPH). This NADH or NADPH, coupled with the 26 electron pairs produced during pyruvate catabolism, would be sufficient to drive the reduction of 66 moles of fumarate to succinate by succinic dehydrogenase. The only requirement for this sequence of events would be a suitable electron transport system.

ACKNOWLEDGMENT

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LITERATURE CITED