APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1983, p. 84-90
0099-2240/83/010084-07$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 45, No. 1

End Products of Glucose Fermentation by *Brochothrix thermosphacta*

FREDERICK H. GRAU

Meat Research Laboratory, Division of Food Research, Commonwealth Scientific and Industrial Research Organization, Cannon Hill, Queenland 4170, Australia

Received 18 May 1982/Accepted 17 September 1982

Anaerobically, *Brochothrix thermosphacta* fermented glucose primarily to L-lactate, acetate, formate, and ethanol. The ratio of these end products varied with growth conditions. Both the presence of acetate and formate and a pH below about 6 increased L-lactate production from glucose. Small amounts of butane-2,3-diol were also produced when the pH of the culture was low (≤5.5) or when acetate was added to the growth medium. Radioactive label from [1-^{14}C]acetate was incorporated into ethanol and L-lactate, implying reversibility of pyruvate-formate lyase. In crude extracts, the following enzymes involved in pyruvate metabolism were demonstrated: lactate dehydrogenase, phosphotransacetylase, acetate kinase, acetaldehyde dehydrogenase (coenzyme A acetylating), ethanol dehydrogenase, pH 6 acetolactate-forming enzyme, and diacetyl (acetoin) reductase. The lactate dehydrogenase did not require fructose-1,6-disphosphate or Mn^{2+} for activity.

Brochothrix thermosphacta is an organism of uncertain taxonomic position and forms a significant component of the spoilage flora of meat and meat products (26). Aerobically, this organism can form cytochromes (5) and use glycerol or carbohydrates as energy sources (8), producing acetate and acetoin (4) as end products. Anaerobically, a fermentable carbohydrate appears necessary for growth, and cytochromes are not produced (L. E. Brownlie, M.Sc. thesis, University of Sydney, Australia, 1969). It is unclear what products are formed from the anaerobic degradation of glucose. McLean and Sulzbacher (20) detected no volatile acids, but found L-lactate and some gas as end products from glucose. Davidson et al. (5) reported that lactate accounts for most of the glucose, no gas is produced, and only trace amounts of acetate and propionate are detectable. Hitchener et al. (11) found that in glucose-limited continuous cultures lactate and ethanol are produced in the approximate ratio of 3:1.

In this study, the end products produced by the anaerobic fermentation of glucose were determined, and the role of some control factors in regulating the formation of these compounds was examined. The presence of some of the key enzymes required for the metabolism of pyruvate to these end products was demonstrated.

MATERIALS AND METHODS

Organism and growth conditions. *B. thermosphacta* ATCC 11509 was grown at 25°C under a nitrogen atmosphere (9) in half-strength mineral salts medium 56 (21) supplemented with 0.3% yeast extract (Difco Laboratories, Detroit, Mich.) and various amounts of glucose, as indicated in the text. Cells used for analysis of enzyme activities or as washed cells were grown in this basal medium containing 7.4 mM glucose. In experiments designed to examine acetate induction of diacetyl (acetoin) reductase, a complex medium (pH 7) was used which contained the following (per liter of distilled water): yeast extract (Oxoid Ltd., London, England), 7.5 g; tryptone (Oxoid), 12.5 g; glucose, 1.33 g; trisodium citrate dihydrate, 5 g; K_2HPO_4, 10 g; $MgSO_4 \cdot 7H_2O$, 1.637 g; $FeSO_4 \cdot 7H_2O$, 0.075 g; and NaCl, sodium acetate, or both, 150 mM. This medium was inoculated with anaerobically grown cells to give an initial turbidity of 8 Klett units (Klett-Summerson colorimeter, no. 66 filter), and the culture was grown under a nitrogen atmosphere for about three generations (final pH, 6.7).

Fermentation of [^{14}C]glucose and [^{14}C]acetate. Basal medium in Thunberg tubes was inoculated with a loopful of an anaerobically grown culture and incubated anaerobically at 25°C for 40 h. In the [^{14}C]glucose fermentation experiments, basal medium contained 3.7 mM [^{14}C]glucose (0.1 mCi/mmol). In the stopper of the Thunberg tube was 0.4 ml of 2 N NaOH as a carbon dioxide trap, and in the body of the Thunberg tube was a smaller tube containing 0.4 ml of 2.5 N H_2SO_4. After incubation, the acid was tipped into the culture, and the Thunberg tube was incubated for a further 2 h to allow any carbon dioxide in the culture to be absorbed into the NaOH trap. In the [^{14}C]acetate fermentation experiments, the basal medium contained 22.2 mM glucose and a total [^{14}C]acetate concentration of 1.22 mM (0.1 mCi/mmol), and no attempt was made to trap carbon dioxide. The radioisotopes used were D-[*U*-^{14}C]glucose (316 mCi/mmol) and [1-

^{14}C]acetate (57 mCi/mmol), both from The Radiochemical Centre, Amersham, England.

Separation and counting of radioactive fermentation products. The NaOH solution was washed from the top portions of the Thunberg tubes, and the radioactivity in a sample was counted. The remaining alkaline solution was treated with $BaCl_2$ and NH_4Cl (final concentration of each, 3.3%) to precipitate trapped carbon dioxide as $BaCO_3$. Precipitated $BaCO_3$ was collected on membrane filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.) and dried before counting. Cells were harvested by centrifugation, washed with 5% trichloracetic acid, and collected on membrane filters. Soluble labeled fermentation end products were separated by the methods of Neish (22) and Dawes et al. (6) for distillation of volatile fatty acids, and ether extraction of nonvolatile acids. Samples of the fermentation supernatant, the material obtained by distillation and ether extraction, and the residues of distillation and ether extraction were chromatographed on silica gel columns (22, 31) with known amounts of unlabeled butyrate, propionate, acetate, formate, lactate, succinate, and ethanol. Samples (0.1 to 1 ml) from the eluates of the silica gel columns and from fractions separated by distillation and ether extraction were added to 10 ml of PCS (Amersham Corp., Arlington Heights, Ill.) scintillation fluid, and the radioactivity was counted in a Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The radioactivity in samples containing chloroform was counted by using both the tritium and ^{14}C windows, and corrections were made for quenching.

Washed-cell suspensions. Cells were harvested by centrifugation (in stoppered centrifuge tubes flushed with nitrogen) from the late-logarithmic phase of an anaerobic culture grown in basal medium plus 7.4 mM glucose and washed with deoxygenated 50 mM potassium phosphate (pH 6.8) containing 5 mM $MgSO_4$ and 1 mM dithiothreitol (DTT). The cells, suspended in deoxygenated 100 mM potassium phosphate containing 5 mM $MgSO_4$ and 5 mM DTT, were placed in Thunberg tubes under a nitrogen atmosphere. After a 15-min incubation at 25°C, glucose and ferrous sulfate were tipped from the side arm to start the reaction and to give a final concentration of 3.6 mM glucose and 0.8 mM $FeSO_4$.

Cell-free extracts. Cells from the late-logarithmic phase of anaerobically grown cultures were washed, suspended, and broken in one of the following buffers (1 g [wet weight] of cells per 4 ml of buffer): 50 mM potassium phosphate (pH 7.0) for the assay of lactate dehydrogenase and NADH oxidase; 50 mM potassium phosphate (pH 6.1)–1 mM $MgCl_2$–0.2 mM thiamine pyrophosphate for the pH 6 acetolactate-forming enzyme (29); 25 mM potassium phosphate (pH 7.0)–25 mM mercaptoethanol–0.1 mM NAD for diacetyl (acetoin) reductase (29); 50 mM Tris-hydrochloride (pH 7.4)–1 mM DTT for acetate kinase; 50 mM Tris-hydrochloride (pH 7.4)–3 mM DTT for both acetaldehyde dehydrogenase (coenzyme A [CoA] acetylating) and ethanol dehydrogenase; and 50 mM Tris-hydrochloride (pH 7.8)–3 mM DTT for phosphotransacetylase.

The cells were broken at 0°C under a nitrogen atmosphere by ultrasonic disruption with a Biosonik IIA probe (Bronwill Scientific Inc., Rochester, N.Y.) operated at full power for 8 to 10 30-s periods with 1-min cooling intervals. Unbroken cells and cell debris were removed by centrifugation at 20,000 × *g* for 20 min. The supernatant was used immediately for the enzyme assays and contained 12 to 15 mg of protein per ml.

Enzyme assays. Pyridine nucleotide oxidation or reduction reactions were measured at room temperature (22 to 23°C) with a recording (Cary 118; Varian Instrument Div., Palo Alto, Calif.) at 340 nm (ε_{340} = 6.22 mM^{-1} cm^{-1}). Reactions without pyridine nucleotides were carried out at 25°C. Specific activities were measured in a range in which linearity with protein concentrations was obtained. Specific activity was defined as micromoles of substrate converted per minute per milligram of protein. Enzymes were assayed with the following reaction mixtures: for lactate dehydrogenase, 100 mM potassium phosphate (pH 6.0)–5 mM sodium pyruvate–0.1 mM NADH; for NADH oxidase, 100 mM potassium phosphate (pH 7.0)–0.1 mM NADH; for phosphotransacetylase (25, 27), 20 mM Tris-hydrochloride (pH 7.8)–50 mM potassium arsenate (pH 7.8)–4 mM acetylphosphate–16 μM CoA–3 mM DTT; for acetate kinase (24), 50 mM Tris-hydrochloride (pH 7.4)–0.775 M sodium acetate (pH 7.4)–10 mM $MgCl_2$–10 mM ATP–0.7 M neutralized hydroxylamine hydrochloride; for pH 6 acetolactate-forming enzyme (29), 50 mM sodium acetate (pH 5.8)–40 mM sodium pyruvate (pH 5.8)–0.5 mM $MnCl_2$–87 μm of thiamine pyrophosphate; for diacetyl (acetoin) reductase (17, 29), 50 mM sodium pyrophosphate–11 mM glycine (pH 9.4)–100 mM butane-2,3-diol–1.75 mM NAD; for ethanol dehydrogenase (1), 85 mM sodium pyrophosphate–19 mM glycine–76 mM semicarbazide hydrochloride (pH 9.0)–0.625 M ethanol–1.75 mM NAD–3 mM DTT; for acetaldehyde dehydrogenase (CoA acetylating) (2, 15), 100 mM glycine–10 mM potassium arsenate (pH 8.8)–100 mM acetaldehyde–0.1 mM CoA–1.75 mM NAD–3 mM DTT.

Analytical methods. Proteins in the cell-free extracts were precipitated with deoxycholate-trichloroacetic acid (23) and determined by the method of Lowry et al. (19). Glucose, L-lactate, and ethanol were assayed enzymatically with assay kits (Calbiochem-Behring Corp., La Jolla, Calif.). Acetate was determined enzymatically by the acetate kinase (Boehringer-Mannheim Corp., New York, N.Y.) method of Rose (24) or with the kit supplied by Boehringer-Mannheim. Formate, butane-2,3-diol, acetoin, acetone, and acetyl phosphate were assayed by the methods of Lang and Lang (16), Keen and Walker (12), Krampitz (14), Lehninger (18), and Stadtman (28), respectively.

RESULTS

[*U*-^{14}C]glucose metabolism. *B. thermosphacta* was grown anaerobically in the presence of 3.7 mM [*U*-^{14}C]glucose until the glucose was exhausted. Only 1.7% of the [^{14}C]glucose was incorporated into acid-insoluble cell material. Although 2.7% of the added ^{14}C was found in the material in the NaOH trap, no counts were detected in the $BaCO_3$ precipitated from the NaOH. This implies that essentially no carbon dioxide was produced during the complete metabolism of 3.7 mM glucose. Four ^{14}C-labeled end products (ethanol, acetate, formate, and

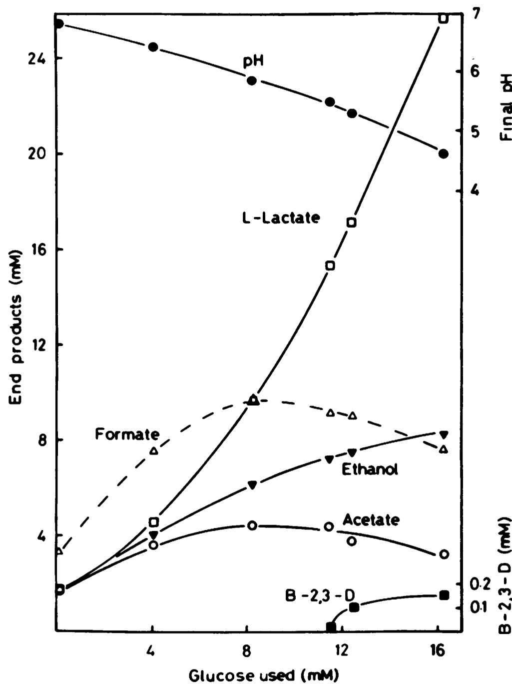

FIG. 1. Changes in end products formed during the fermentation of increasing amounts of glucose. Symbols: ●, pH; □, L-lactate; △, formate; ○, acetate; ▼, ethanol; ■, butane-2,3-diol.

lactate) were identified in the culture supernatant by silica-gel column chromatography and fractional distillation. The percentages of ^{14}C found in these end products were: ethanol, 22.2%; acetate, 22.3%; formate, 21.6%; and lactate, 25.9%. No ^{14}C-labeled acetone, acetoin, propionate, butyrate, or succinate was detected. Of the ^{14}C added as glucose, 96.4% was recovered in identifiable fractions.

Changes in end products with increasing glucose. The relationship between the proportions of the end products and the amount of glucose consumed by cultures growing anaerobically is summarized in Fig. 1. In basal medium without added glucose, about equimolar proportions of lactate, ethanol, and acetate were formed. The molar proportion of formate produced was approximately equal to that of the sum of acetate and ethanol. Since only 0.1 to 0.2 mM glucose was detected in the basal medium, these end products must be formed from other constituents (presumably carbohydrates) in the yeast extract. As increasing amounts of glucose were added to the medium, the concentrations of formate and acetate rose to a maximum at around 8.25 mM glucose used, or at about pH 5.9. With greater glucose consumption, both acetate and formate concentrations declined. Although ethanol production increased with increasing glucose consumption, less of the glucose was converted to ethanol at higher glucose concentrations. On the other hand, with increasing glucose concentrations, more glucose was converted to lactate. It appeared that at pH values less than 6, acetate, formate, and ethanol productions were markedly reduced and the production of lactate increased. Only at pH values ≤5.5 was butane-2,3-diol detected, and then it accounted for only about 1% of the glucose used.

[1-^{14}C]acetate incorporation. The concentration of acetate in culture supernatants was low at high glucose concentrations (Fig. 1). This suggested that acetate was able to be metabolized, possibly to ethanol and, since the formate concentration was also low, probably also to lactate. *B. thermosphacta*, therefore, was grown anaerobically in basal medium containing 22.2 mM glucose and [1-^{14}C]acetate (1.22 mM), and when growth had ceased (final pH, 4.61), ^{14}C incorporation into acid-insoluble cellular material and end products was examined. Over 40% of the recovered ^{14}C was found in ethanol, and 26% was found in lactate, indicating that a considerable amount of acetate was metabolically converted (Table 1).

Effect of pH on glucose metabolism by washed cells. Because lactate production from glucose increased and production of the other end products decreased as the pH of the culture fell (Fig. 1), the effect of pH on glucose metabolism by washed cells was examined. The ability of washed cells to produce formate, acetate, and ethanol from glucose was oxygen sensitive. Lactate, but little or no ethanol and formate, was produced from glucose when washed cells were prepared in the absence of reducing agents, even though attempts were made to reduce access of air to the cells. Therefore, 1 mM DTT was added

TABLE 1. Incorporation of [1-^{14}C]acetate into fermentation products by anaerobically growing *B. thermosphacta*[a]

Product	cpm (%)	μmol/ml	Sp act[b]
Before growth (Acetate)	100	1.22	1.31×10^5
After growth			
Cells	3		
Acetate	18	4.2	7×10^3
Ethanol	40	8.0	8×10^3
Lactate	26	26.0	1.6×10^3

[a] *B. thermosphacta* was anaerobically grown at 25°C in basal medium containing 22.2 mM glucose and 1.22 mM [1-^{14}C]acetate. After growth ceased (40 h), the amount of products and their radioactivity was determined as described in the text.

[b] Specific activity is counts per minute per micromole.

TABLE 2. Effect of pH on the anaerobic metabolism of glucose by washed cells[a]

Product formed	Concn (mM) at:		
	pH 5.4	pH 6.6	pH 7.4
L-lactate	4.6	3.5	3.5
Ethanol	2.0	2.4	2.3
Acetate	0.3	1.1	1.1
Formate	2.0	3.4	3.2

[a] Washed cells were incubated with 3.6 mM glucose–100 mM potassium phosphate–5 mM $MgSO_4$–5 mM DTT–0.8 mM ferrous sulfate for 1 h at 25°C. All of the glucose was consumed.

to the buffer used to wash the cells, and 5 mM DTT and 0.8 mM ferrous sulfate were included in the reaction mixture to ensure a low redox potential (13). All of the glucose (3.6 mM) was consumed, and no butane-2,3-diol were detected (<0.01 mM) in the end products. There was no significant difference in end products at pH 6.6 and 7.4 (Table 2). At pH 5.4, however, about a third more lactate was formed, and there was a corresponding reduction in the amounts of the other end products. The low acetate-to-ethanol ratio may be the result of incubating the cells with DTT and ferrous sulfate to ensure a low redox potential.

Effects of acetate and formate on end products. Although incubation of cells at a low pH (5.4) did change the end products formed from glucose, this change was small relative to that seen with growing cultures (Fig. 1). The role of acetate and formate in influencing end products was therefore examined in growing cultures where the pH did not fall below 6.6. *B. thermosphacta* was grown anaerobically in basal medium with 1.85 mM added glucose (total glucose, 2.1 mM) and with up to 75 mM added sodium acetate, sodium formate, or both. When both acetate and formate were added to the growth medium, these compounds were added in the molar ratio of 1:2. After growth had ceased, the final pH values of the growth media were 6.63 to 6.74, and no residual glucose was detected.

Both acetate and formate brought about changes in the production of lactate and ethanol (Fig. 2). Formate was more effective than acetate in increasing the yield of lactate, and acetate plus formate was more effective than either alone. Acetate alone caused a moderate reduction in ethanol formation. Ethanol formation was even further reduced by formate or by formate plus acetate. Small amounts of butane-2,3-diol were produced when acetate or acetate plus formate was added to the culture media. No butane-2,3-diol was detected in cultures grown without added acetate, even when 75 mM formate was present.

Enzymes associated with the conversion of pyruvate to end products. Table 3 lists the specific activities of the enzymes examined in cell-free extracts prepared from cells anaerobically grown in basal medium with 7.4 mM glucose.

When crude extracts were assayed in the direction of L-lactate oxidation with NAD, no activity was detected with 5 mM lactate and 0.1 mM NAD in phosphate buffer at pH 6.0 or 7.9. However, there was considerable enzyme activity when lactate dehydrogenase was measured in the reverse direction of pyruvate reduction with NADH. The pH optimum was between 6.0 and 6.5 in both potassium phosphate buffer and imidazole-hydrochloride buffer. Over the pH range of 6.0 to 7.9, the rate of pyruvate reduction was 25 to 30% faster in phosphate buffer than in imidazole buffer. At pH 7.0 the reaction rate was about 70%, and at pH 7.9 about 25%, of the rate at pH 6.0. The rate of pyruvate reduction by the crude extract was not significantly changed by an 18-h dialysis against three changes of 200 volumes of potassium phosphate buffer (pH 7). Neither 1 mM fructose-1,6-diphosphate (Fru-1,6-P_2) nor 1 mM manganese sulfate separately or together increased the enzyme activity of the dialyzed extract. Fruc-1,6-P_2 had no significant effect on either the V_{max} or the K_m for pyruvate when measured at pH 6.0 with phosphate buffer or at pH 7.5 with imidazole buffer. At pH 6.0 in phosphate buffer, K_m for pyruvate was 1.26 mM (range, 1.04 to 1.33 mM) and the V_{max} was 2.85 μmol of pyruvate per min per mg of total protein. At pH 7.5 in imidazole buffer, the K_m was increased to 3.2 mM and the

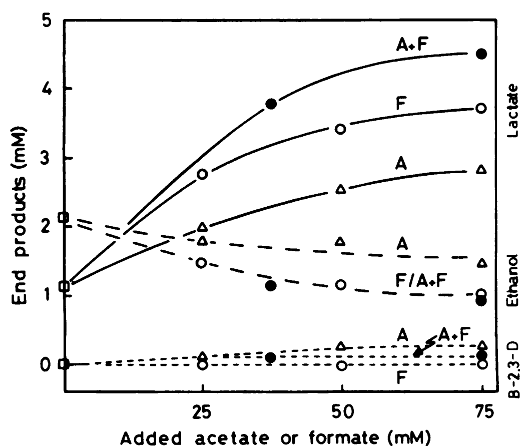

FIG. 2. Effect of acetate (A) or formate (F) or both (A + F) on end products formed by anaerobically growing *B. thermosphacta*. To the culture media was added: acetate (△); formate (○); or acetate plus formate (1:2 mole ratio) (●). After growth ceased, L-lactate (——), ethanol (—), and butane-2,3-diol (-----) were measured.

TABLE 3. Specific activities of enzymes involved in pyruvate metabolism

Enzyme	Sp act[a]
Lactate dehydrogenase	2.75
NADH oxidase	<0.01
Phosphotransacetylase	0.76
Acetate kinase	3.29
Acetaldehyde dehydrogenase (CoA acetylating; NAD)	0.64
Ethanol dehydrogenase (NAD)	2.19
Ethanol dehydrogenase (NADP)	<0.01
pH 6 acetolactate-forming	0.43
Diacetyl (acetoin) reductase	0.39

[a] Specific activity is micromoles of substrate converted per minute per milligram of protein.

V_{max} was reduced to 1.2 to 1.3 μmol per min per mg of protein.

Phosphotransacetylase was measured by using the CoA-dependent arsenolysis of acetyl phosphate. When CoA was not added to the reaction mixture, the rate of breakdown of acetyl phosphate was only 17% of that in the complete assay reaction. In the absence of arsenate alone, the rate of acetyl phosphate hydrolysis was reduced to 6%. Acetate kinase was measured in the direction of acetyl phosphate formation. Treatment of a diluted crude extract (800 μg of protein/ml) with an approximately equal volume of Dowex 1-Cl^- (Dow Chemical Co., Midland, Mich.) to remove CoA brought about only a 20% reduction in acetate kinase activity, indicating that the major enzyme activity assayed was acetate kinase rather than acetyl-CoA synthetase.

In 50 mM sodium phosphate buffer, the activity of the enzyme forming acetolactate from pyruvate was greater at pH 5.8 than at either pH 6.4 or 7.7. At pH 5.8, enzyme activity was about 60% greater in 50 mM sodium acetate buffer than in phosphate buffer. This is consistent with the presence in the crude extract of the pH 6 acetolactate-forming enzyme.

Acetate in the growth medium caused some increase in the amount of diacetyl (acetoin) reductase formed. In cell-free extracts prepared from cells grown in complex medium (initially containing 0.9 mM acetate), the specific activity of the diacetyl (acetoin) reductase was 0.21 μmol per min per mg of protein. The addition of 25 mM sodium acetate more than doubled the amount of the enzyme formed. There was no further increase in specific activity when the complex medium contained 50 to 150 mM sodium acetate and the pH of the growth media was kept between 7.0 and 6.7.

Although NAD-linked ethanol dehydrogenase was highly active in the crude extracts, no activity could be detected with NADP. In contrast, acetaldehyde dehydrogenase (CoA acetylating) showed activity with both NAD and NADP. The activity with NADP was about 80% of that observed with NAD. The decrease in reaction rate with time was less with NADP presumably because, whereas NADH could have been reoxidized, NADPH was not used by the alcohol dehydrogenase present. In the absence of added CoA, the rate of acetaldehyde oxidation (with either NAD or NADP) was reduced to less than 3%. Arsenate increased enzyme activity about 60% and allowed the reaction to continue longer, presumably by allowing the regeneration of CoA via the endogenous phosphotransacetylase. In Tris-hydrochloride buffer at pH 7.2, enzyme activity was only about 5 to 10% of the activity in glycine buffer at pH 8.8 and ceased within about 20 s.

DISCUSSION

As very little, if any, carbon dioxide was produced anaerobically from D-[U-^{14}C]glucose when the pH of the culture was between 7.0 and 6.4, neither the pentose pathway nor the heterofermentative phosphoketolase pathway can be a major means of glucose degradation. The high cell yield obtained by Hitchener et al. (11) and the low ATP yield from glucose by the Entner-Duodoroff pathway imply that this pathway is unlikely to be a major pathway of glucose metabolism. High activities of hexokinase and fru-1,6-P_2 aldolase have been detected in *B. thermosphacta* (3). It is, therefore, most likely that, under anaerobic conditions, glucose is fermented principally by the Embden-Meyerhoff-Parmas pathway to pyruvate, and thence to lactate, acetate, ethanol, and formate as the major end products.

In growing cultures, as increasing amounts of glucose were consumed and the pH of the growth medium decreased, increasing amounts of lactate and decreasing amounts of acetate, ethanol, and formate were produced. In homofermentative lactobacilli and streptococci, a change from mixed-acid fermentation at low glucose concentrations to lactic fermentation at high glucose concentrations is usually associated with the lactic dehydrogenase being Fru-1,6-P_2 regulated (7). However, *B. thermosphacta* lactic dehydrogenase did not appear to be under Fru-1,6-P_2 regulation. After dialysis of cell-free extracts, no requirement for or stimulation by Fru-1,6-P_2 could be shown in phosphate or imidazole buffer over the pH range of 6.0 to 7.9. The pH optimum of 6.0 to 6.5 for lactic dehydrogenase and the increased lactate production by washed cells at pH values lower than 6.0 are consistent with increased lactate formation as the pH of the culture falls below 6.0.

Acetate and formate, however, probably play

a more important role in the diversion of pyruvate to different end products. Even in cultures where the pH did not fall below 6.6, the addition of acetate and formate altered the end products formed from pyruvate. When 12.5 mM acetate and 25 mM formate were added to growing cultures, the lactate formed increased almost fourfold, and the ethanol produced was reduced by about half. In growing cultures without added acetate or formate, net production of acetate and formate ceased, and formation of ethanol was considerably reduced at pH 5.8 to 5.9 when the metabolically produced acetate and formate levels were 4.4 and 9.7 mM, respectively. Since bacterial cells are normally more permeable to undissociated formic and acetic acids than to the ionized anions, lowering the pH of the growth medium can be expected to increase the effect of these acids in altering end products. It therefore appears that as acetate and formate accumulate in growing cultures, particularly when the pH falls, these acids reduce the amount of glucose that can be diverted through the pyruvate-formate lyase pathway, and an increasing amount of glucose is catabolized to lactate.

Acetate also plays a role in the production of the small amount of butane-2,3-diol formed. When the pH of the growing cultures was above 6.6, butane-2,3-diol was detected only in the presence of added acetate, and this addition of acetate doubled the amount of diacetyl (acetoin) reductase formed. Furthermore, the activity of the pH 6 acetolactate-forming enzyme was higher when assayed in the presence of acetate. These results are similar to those obtained for the butane-2,3-diol-forming system of *Aerobacter aerogenes* (17), in which acetate, particularly at low pH values, acts as a coordinate inducer of the three enzymes required for the production of butane-2,3-diol from pyruvate, acts as an activator of the pH 6 acetolactate-forming enzyme, and favours butane-2,3-diol formation by diacetyl (acetoin) reductase. However, compared with *A. aerogenes*, *B. thermosphacta* appeared to produce less of the latter two enzymes, and the induction by acetate was less.

The experiments with [1-^{14}C]acetate show that acetate can enter the metabolic pool and can be reduced to ethanol. The enzymes necessary for this (acetate kinase, phosphotransacetylase, acetaldehyde dehydrogenase [CoA acetylating], and ethanol dehydrogenase) were all present in cell-free extracts at high activities. In addition, [1-^{14}C]acetate was incorporated into lactate, presumably by condensation of [1-^{14}C]acetyl-CoA with formate by the reverse operation of pyruvate-formate lyase. Of the total ^{14}C originally in acetate, 26% was recovered as lactate, and the specific activity of the lactate was about 20% of that of ethanol and acetate. The reversibility of pyruvate-formate lyase has been shown in cell-free extracts of clostridia (30) and in growing cultures of *Escherichia coli* and *Aerobacter cloacae* (10).

ACKNOWLEDGMENTS

I thank P. B. Vanderlinde for excellent technical assistance.

This work was supported by the Australian Meat Research Committee.

LITERATURE CITED

1. **Bergmeyer, H. U.** 1974. Methods of enzymatic analysis, 2nd ed., p. 428–429. Academic Press, Inc., New York.
2. **Clark, D. P., and J. E. Cronan, Jr.** 1980. Acetaldehyde coenzyme A dehydrogenase of *Escherichia coli*. J. Bacteriol. **144**:179–184.
3. **Collins-Thompson, D. L., T. Sørhaug, L. D. Witter, and Z. J. Ordal.** 1972. Taxonomic consideration of *Microbacterium lacticum*, *Microbacterium flavum*, and *Microbacterium thermosphactum*. Int. J. Syst. Bacteriol. **22**:65–72.
4. **Dainty, R. H., and C. M. Hibbard.** 1980. Aerobic metabolism of *Brochothrix thermosphacta* growing on meat surfaces and in laboratory media. J. Appl. Bacteriol. **48**:387–396.
5. **Davidson, C. M., P. Mobbs, and J. M. Stubbs.** 1968. Some morphological and physiological properties of *Microbacterium thermosphactum*. J. Appl. Bacteriol. **31**:551–559.
6. **Dawes, E. A., D. J. McGill, and M. Midgley.** 1971. Analysis of fermentation products, p. 53–215. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 6A. Academic Press, Inc., London.
7. **Garvie, E. I.** 1980. Bacterial lactate dehydrogenases. Microbiol. Rev. **44**:106–139.
8. **Grau, F. H.** 1979. Nutritional requirements of *Microbacterium thermosphactum*. Appl. Environ. Microbiol. **38**:818–820.
9. **Grau, F. H.** 1980. Inhibition of the anaerobic growth of *Brochothrix thermosphacta* by lactic acid. Appl. Environ. Microbiol. **40**:433–436.
10. **Higgins, T. E., and M. J. Johnson.** 1970. Pathways of anaerobic acetate utilization in *Escherichia coli* and *Aerobacter cloacae*. J. Bacteriol. **101**:885–891.
11. **Hitchener, B. J., A. F. Egan, and P. J. Rogers.** 1979. Energetics of *Microbacterium thermosphactum* in glucose-limited continuous culture. Appl. Environ. Microbiol. **37**:1047–1052.
12. **Keen, A. R., and N. J. Walker.** 1973. Separation of diacetyl, acetoin, and 2,3-butylene glycol by ion-exchange chromatography. Anal. Biochem. **52**:475–481.
13. **Knappe, J., and H. P. Blaschkowski.** 1975. Pyruvate-formate lyase from *Escherichia coli* and its activation system. Methods Enzymol. **41**:508–518.
14. **Krampitz, L. O.** 1957. Preparation and determination of acetoin, diacetyl, and acetolactate. Methods Enzymol. **3**:277–283.
15. **Lamed, R., and J. G. Zeikus.** 1980. Glucose fermentation pathway of *Thermoanaerobium brockii*. J. Bacteriol. **141**:1251–1257.
16. **Lang, E., and H. Lang.** 1972. Spezifische Farbreaktion zum direkten Nachweis der Ameisensäure. Z. Anal. Chem. **260**:8–10.
17. **Larsen, S. H., and F. C. Størmer.** 1973. Diacetyl (acetoin) reductose from *Aerobacter aerogenes*. Kinetic mechanism and regulation by acetate of the reversible reduction of acetoin to 2,3-butanediol. Eur. J. Biochem. **34**:100–106.
18. **Lehninger, A. L.** 1957. Colorimetric determination of acetoacetate. Methods Enzymol. **3**:283–285.
19. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
20. **McLean, R. A., and W. L. Sulzbacher.** 1953. Microbacterium thermosphactum, spec. nov; a nonheat resistant

bacterium from fresh pork sausage. J. Bacteriol. **65**:428–433.

21. **Monod, J., G. Cohen-Bazire, and M. Cohn.** 1951. Sur la biosynthése de la β-galactosidase (lactase) chez *Escherichia coli*. La spécificité de l'induction. Biochim. Biophys. Acta **7**:585–599.
22. **Neish, A. C.** 1952. Analytical methods for bacterial fermentations, 2nd revision. Report no. 46-8-3. National Research Council of Canada, Saskatoon, Saskatchewan.
23. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. Anal. Biochem. **83**:346–356.
24. **Rose, I. A.** 1955. Acetate kinase of bacteria (acetokinase). Methods Enzymol. **1**:591–595.
25. **Shimizu, M., T. Suzuki, K. Kameda, and Y. Abiko.** 1969. Phosphotransacetylase of *Escherichia coli* B, purification and properties. Biochim. Biophys. Acta **191**:550–558.
26. **Sneath, P. H. A., and D. Jones.** 1976. *Brochothrix*, a new genus tentatively placed in the family *Lactobacillaceae*. Int. J. Syst. Bacteriol. **26**:102–104.
27. **Stadtman, E. R.** 1955. Phosphotransacetylase from *Clostridum kluyveri*. Methods Enzymol. **1**:596–599.
28. **Stadtman, E. R.** 1957. Preparation and assay of acetyl phosphate. Methods Enzymol. **3**:228–231.
29. **Störmer, F. C.** 1975. 2,3-Butanediol biosynthetic system in *Aerobacter aerogenes*. Methods Enzymol. **41B**:518–533.
30. **Thauer, R. K., F. H. Kirchniawy, and K. A. Jungermann.** 1972. Properties and function of the pyruvate-formatelyase reaction in Clostridiae. Eur. J. Biochem. **27**:282–290.
31. **Varner, J. E.** 1957. Chromatographic analyses of organic acids. Methods Enzymol. **3**:397–403.