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Metabolism of Pyruvate and Citrate in Lactobacilli

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Abstract

Lactobacillus acidophilus, *L. bulgaricus*, *L. casei*, *L. delbrueckii*, *L. lactis* and *L. plantarum* contained a pyruvate oxidase for the oxidation of pyruvate to acetyl phosphate and acetate. The presence of an acetate kinase converted the acetyl phosphate to acetate. *L. casei* and *L. plantarum* produced lactate and acetoin, in addition to acetate, under the conditions used while *L. casei* also produced diacetyl. *L. casei* and *L. plantarum* were the only species to utilize citrate. *L. helveticus* and *L. helveticus* subsp. *jugurti* did not utilize pyruvate under the conditions used.

Introduction

Metabolism of Pyruvate

Pyruvate is a key intermediate in the metabolism of many lactic acid bacteria and can be converted into a variety of end products, such as lactic acid, formic acid, acetic acid, acetaldehyde, ethanol, acetoin, diacetyl and butane-2,3-diol.

Homofermentative lactobacilli utilize the glycolytic pathway for the production of energy and convert at least 85% of fermentable carbohydrate to lactic acid; the remaining 15% being converted to CO_2, ethanol or acetic acid or both (Christensen *et al.* 1958; Dirar and Collins 1972).

The oxidation of pyruvate to acetyl phosphate and acetate by *L. delbrueckii* was shown by Hager *et al.* (1954) to be catalysed by a flavin-linked pyruvate oxidase. Lloyd *et al.* (1978) showed that *L. bulgaricus* also used pyruvate oxidase to catalyse the formation of acetyl phosphate from pyruvate. These authors also found that *L. bulgaricus* possessed both acetate kinase and phosphate acetyltransferase activities and converted acetyl phosphate to acetate. These results were extended in this investigation to include a number of strains of *L. bulgaricus*.

In order to show that pyruvate oxidase was the enzyme responsible for the conversion of pyruvate to acetate in *L. bulgaricus* it was necessary to demonstrate that acetyl phosphate and acetate were formed in a ratio of 1 : 1:

$$2\ \text{Pyruvate} + O_2 + P_i \rightarrow \text{acetyl phosphate} + \text{acetate} + 2CO_2 + H_2O\ .$$

However, under normal conditions, acetyl phosphate is readily converted to acetate by acetate kinase (EC 2.7.2.1), acid phosphatase (EC 3.1.3.2) and by non-enzymic hydrolysis. Cell-free extracts were therefore dialysed to remove ADP (thus inactivating acetate kinase) and sodium fluoride was added to the reaction mixture to inhibit acid phosphatase activity.

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The oxidation of pyruvate in other homofermentative lactobacilli has not been extensively studied. However, it has been shown that (i) lipoic acid and CoA were not involved in the production of acetate in *L. plantarum* (Dirar and Collins 1973); (ii) the metabolism of pyruvate in *L. plantarum* produced acetoin as a major product (Rowatt 1951; Nossal 1952) through the enzymic decarboxylation of α-acetolactate (Hunt and Nossal 1954); and (iii) the metabolism of pyruvate in *L. casei* produced α-acetolactate which was then converted to diacetyl and acetoin (Branen and Keenan 1972; Gardenas *et al.* 1980).

Metabolism of Citrate

The fermentation of citrate is a property of species of lactobacilli such as *L. casei*, *L. plantarum* and *L. brevis* (Fryer 1970). However, little has been reported about citrate fermentation in species such as *L. helveticus*, *L. bulgaricus* and *L. acidophilus*.

L. plantarum has been shown to produce increased levels of acetoin or diacetyl or both when grown in the presence of citrate (Christensen and Pederson 1958; Drinan *et al.* 1976). Citrate has also been found to stimulate the growth of *L. casei* (Branen and Keenan 1970) and can be used by this organism as a growth substrate in the absence of fermentable carbohydrate (Campbell and Gunsalus 1944). The utilization of citrate by growing cultures of *L. casei* resulted in the formation of CO_2, acetic acid, formic acid, lactic acid and small amounts of ethanol and acetoin (Campbell and Gunsalus 1944). The fermentation balances suggested a phosphoroclastic cleavage of pyruvate, derived from citrate, to form formic acid and acetyl CoA. The acetyl CoA could be converted to acetate to provide the energy for growth. Campbell and Gunsalus (1942) have shown that the amount of formate produced from citrate decreased as the pH of the growth medium decreased and Fryer (1970) showed that the yield of formate from citrate was never greater than 35% of that which was theoretically possible.

As pyruvate and citrate have been postulated to be key intermediates in the production of flavour compounds in fermented dairy products, the aim of the present investigation was to study the metabolism of these compounds in several species of lactobacilli.

Materials and Methods

Bacteria

Growth conditions

The organisms used in this investigation were *L. bulgaricus*, strains 1006, 1243, 1373, 1489, 1978, and 1979; *L. delbrueckii* 231; *L. helveticus*, strains 30, 257, 261, 384, 766 and 1829; *L. helveticus* subsp. *jugurti* YBI; *L. lactis* 270; *L. acidophilus* 1748; *L. casei* 151; and *L. plantarum* 343. *L. helveticus* subsp. *jugurti* YBI was obtained from the Dairy Research Laboratory, Division of Food Research, CSIRO, Highett, Vic. and all other organisms were obtained from the National Collection of Dairy Organisms (NCDO) at the National Institute for Research in Dairying, Reading, England.

The cultures were obtained freeze-dried in ampoules and were maintained as stock at 5°C in sterile skim milk, containing litmus and calcium carbonate, and subcultured at 3-monthly intervals. Cultures in use were maintained by daily subculture into sterile skim milk and inoculated, as required, into MRS broth (de Man *et al.* 1960).

L. helveticus subsp. *jugurti* YBI, which did not grow in MRS broth, was grown in M17 broth (Terzaghi and Sandine 1975). *L. casei* and *L. plantarum* were grown at 30°C and all other species at 37°C. When large quantities of cells were required for the preparation of cell-free extracts, the growth medium was maintained at pH 6·2 by the addition of 10 M NaOH. This addition was controlled by a magnetic valve (Radiometer, model MNV1) connected to a titrator (Radiometer, TTT11b). Cells were harvested by centrifugation at 4 000 *g* for 10 min at 4°C in a Sorvall refrigerated centrifuge (Model RC2-B), washed twice and resuspended in 0·9% (w/v) NaCl.

Preparation of cell-free extracts

Cell-free extracts were prepared by extrusion of the cell suspension through a French pressure cell (French and Milner 1955; Vanderheiden *et al.* 1970) at a constant pressure of 155 MPa (1575 kg/sq.cm).

Unbroken cells and cell debris were removed by centrifugation at 30 000 *g* for 30 min at 4°C. Where indicated, cell-free extracts were dialysed against 20 mM KH_2PO_4 buffer, pH 7·0, for 48 h.

Respiratory Studies

All incubations were carried out in single side-arm Warburg flasks at either 30°C (for *L. casei* and *L. plantarum*) or 37°C (for all other species). For studies using resting cells, the reaction mixture (3·0 ml) contained (in μmoles): KH_2PO_4 (adjusted to the appropriate pH with 1M NaOH), 300; $MgCl_2$, 5; thiamine pyrophosphate (TPP), 0·1; cells (approximately 20 mg dry weight); and either pyruvate, 60, acetaldehyde, 60 or citrate, 100 (unless otherwise stated). For studies using cell-free extracts, the reaction mixture (3·0 ml) contained (in μmoles): KH_2PO_4 (adjusted to the appropriate pH with 1 M NaOH), 200; $MgCl_2$, 10; TPP, 0·2; FAD, 0·1; 0·5 ml of cell-free extract (approximately 7·5 mg protein); and either pyruvate, 60, acetaldehyde, 60 or citrate 100 (unless otherwise stated).

The reactions were initiated by the addition of whole cells or cell-free extracts from the side-arm of the reaction flasks and stopped by rapidly cooling the flasks in ice-water (for whole cells) or by the addition of 0·1 ml of 10 M HCl (for cell-free extracts). The contents of each flask were then centrifuged at 5 000 *g* for 5 min to remove either the whole cells or the precipitated protein and the supernatants were stored at −20°C until analysed for residual pyruvate, citrate or acetaldehyde and for products arising from the metabolism of these compounds.

Analytical Methods

O_2- uptake and CO_2-evolution were estimated manometrically as described by Umbreit *et al.* (1957). The values for CO_2 evolution were corrected for the retention of CO_2 in solution as bicarbonate.

Pyruvate was estimated by the enzymic method of Von Korff (1969).

Citrate was determined using a Boehringer Mannheim kit (Cat. No. 139076).

Acetate was measured as volatile acid by the method of Thomas *et al.* (1979). As a small amount of volatile acid was formed from the residual pyruvate during distillation, this was subtracted from the total volatile acid in the distillate.

Acetaldehyde, ethanol, acetoin and diacetyl were separated by gas chromatography on Porapack Q, as described by Coventry *et al.* (1978) and estimated by measuring areas under the peaks using propanol as an internal standard.

Acetyl phosphate was estimated by the method of Lipmann and Tuttle (1945).

D- *and* L-*lactate* were estimated by the method of Noll (1974).

Protein was estimated by the method of Lowry *et al.* (1951).

Dry weight of cells was determined by reference to a standard curve relating dry weight with absorbance at 650 nm.

Results

Pyruvate Oxidation

Lactobacillus bulgaricus

Because the cells were able to obtain pyruvate (present in the incubation mixtures) without the concomitant production of NADH, they were free to metabolize pyruvate by enzymes other than lactate dehydrogenase. As shown in Table 1, resting cells and cell-free extracts of all the strains of *L. bulgaricus* tested utilized pyruvate according to the reaction sequence:

$$2\ \text{Pyruvate} + O_2 \rightarrow 2\ \text{acetate} + 2CO_2\ .$$

No acetyl phosphate was detected under these conditions.

However, dialysed cell-free extracts of all strains were able to convert pyruvate to acetyl phosphate, but with the exception of strain 1373, the ratio of acetyl phosphate to acetate was not 1 : 1 (Table 2). This suggested that acetyl phosphate was an intermediate in the formation of acetate. Moreover, since acetaldehyde was not oxidized to acetate and arsenite did not inhibit the utilization of oxygen (data not shown) it is unlikely that acetate (or acetyl phosphate) was formed from pyruvate by either a yeast type pyruvate decarboxylase reaction (pyruvate → acetaldehyde → acetate) or by the pyruvate dehydrogenase system.

The inability to completely inhibit the enzymic and non-enzymic hydrolysis of acetyl phosphate was probably responsible for the less than theoretical recovery of acetyl phosphate.

Table 1. Formation of acetate from pyruvate by whole cells and cell-free extracts of *L. bulgaricus*, *L. casei* and *L. plantarum*

Cells were grown in MRS broth for 16 h at 37°C (*L. bulgaricus*) or 30°C (*L. casei* and *L. plantarum*), harvested, washed, and cell-free extracts prepared, as described under Methods. The incubation mixture for whole cells (3·0 ml) contained (in μmoles): KH_2PO_4 (pH 5·5), 300; $MgCl_2$, 5; TPP, 0·1; sodium pyruvate, 60 (approx.) and 20 mg (dry weight) of cells. The incubation mixture for cell-free extracts (3·0 ml) contained (in μmoles): KH_2PO_4 (pH 5·5), 200; $MgCl_2$, 10; TPP, 0·2; FAD, 0·1; sodium pyruvate, 60 (approx.) and 0·5 ml of cell-free extract (approx. 3 mg protein). Reactions were carried out in Warburg flasks at 37°C (*L. bulgaricus*) or 30°C (*L. casei* and *L. plantarum*). Analytical procedures were as described under Methods

Strain	Substrate utilized[A]		CO_2	Products formed[A]				
	Pyruvate	O_2	evolved[A]	Acetate	Acetoin	Diacetyl	Lactate	Lactate isomer
				L. bulgaricus				
1006								
Whole cells	0·25	0·14	0·28	0·35	—	—	—	—
Cell-free extract	4·26	2·09	3·87	3·69	—	—	—	—
1243								
Whole cells	0·63	0·32	0·64	0·69	—	—	—	—
Cell-free extract	2·37	1·24	2·54	2·40	—	—	—	—
1373								
Whole cells	0·19	0·10	0·19	0·20	—	—	—	—
Cell-free extract	4·06	2·25	4·46	4·38	—	—	—	—
1489								
Whole cells	0·54	0·27	0·54	0·56	—	—	—	—
Cell-free extract	5·57	2·45	4·95	4·83	—	—	—	—
1978								
Whole cells	0·40	0·19	0·38	0·36	—	—	—	—
Cell-free extract	5·03	2·43	4·70	4·63	—	—	—	—
				L. casei				
151								
Whole cells	1·47	0·09	1·09	0·49	0·35	0·02	0·27	D
Cell-free extract	1·93	0·39	1·91	0·65	0·34	0·29	0·21	D
				L. plantarum				
343								
Whole cells	1·62	0·33	1·34	0·88	0·29	Nil	0·15	D+L
Cell-free extract	2·19	0·76	1·97	1·86	0·21	Nil	0·16	D+L

[A] Whole cells: μmoles per milligram dry weight of cells per hour; cell-free extracts: μmoles per milligram of protein per hour.

Lactobacillus acidophilus, L. delbrueckii *and* L. lactis

Whole cells and cell-free extracts of *L. acidophilus* 1748, *L. delbrueckii* 213 and *L. lactis* 270 oxidized pyruvate according to the above reaction sequence observed for *L. bulgaricus* (Table 1) and acetyl phosphate was formed from pyruvate by dialysed cell-free extracts (Table 2). As with *L. bulgaricus*, these species of lactobacilli did not form acetate from acetaldehyde or from pyruvate via the pyruvate dehydrogenase system.

Lactobacillus helveticus

Whole cells and cell-free extracts of all strains of *L. helveticus* tested, were unable to utilize pyruvate under the conditions used for *L. bulgaricus*. Varying the pH of the incubation mixture, between 6·0 and 8·0, also had no effect on the utilization of pyruvate by this species.

Lactobacillus casei *and* L. plantarum

The oxidation of pyruvate by *L. casei* 151 and *L. plantarum* 343 differed from the strains of *L. acidophilus, L. bulgaricus, L. delbrueckii* and *L. lactis* investigated, in that significant amounts of lactate and acetoin were formed in addition to acetate (Table 1). *L. casei* 151 also formed diacetyl. Treatment of the cell-free extract to remove CoA (Stadtman 1950; Chantrene and Lipmann 1950) did not reduce diacetyl formation, nor did the addition of CoA increase the formation of diacetyl by *L. casei* 151. Dialysed cell-free extracts of *L. casei* 151 did not metabolize pyruvate even when TPP, FAD and pyridoxalamine were added to the incubation mixture. The acetoin formed by *L. casei* 151 and *L. plantarum* 343 in the present study was not formed by a yeast-type condensation of acetaldehyde with the acetaldehyde–TPP complex (Juni 1952*b*; Chuang and Collins 1968) since the addition of acetaldehyde with pyruvate, did not increase the level of acetoin produced by these organisms (data not shown).

Table 2. Formation of acetate and acetyl phosphate from pyruvate (in the presence of fluoride) by dialysed cell-free extracts of some species of lactobacilli

Cells were grown in MRS broth for 16 h at 37°C, harvested, washed, and cell-free extracts prepared, as described under Methods. The incubation mixture (3·0 ml) contained (in μmoles): KH_2PO_4 (pH 6·5), 200; $MgCl_2$, 10; TPP, 0·2; FAD, 0·1; sodium pyruvate, 60 (approx.); NaF, 100; and 0·5 ml of cell-free extract (3 mg protein), previously dialysed against 20 mM KH_2PO_4 (pH 7·0) for 48 h. Reactions were carried out in Warburg flasks for 2 h at 37°C. Analytical procedures were as described under Methods

Strain	Substrate utilized[A]		CO_2 evolved[A]	Products formed[A]	
	Pyruvate	O_2		Acetate	Acetyl phosphate
			L. acidophilus		
1748	0·68	0·29	0·58	0·30	0·22
			L. bulgaricus		
1006	2·27	1·19	2·26	1·77	0·72
1243	4·00	1·89	3·82	2·76	1·19
1373	2·32	1·11	2·19	1·11	1·04
1489	1·80	0·88	1·83	1·55	0·10
1978	0·87	0·35	0·59	0·59	0·17
			L. delbrueckii		
243	0·89	0·40	0·79	0·54	0·37
			L. lactis		
270	6·45	3·28	6·40	4·04	2·21

[A] Units: μmoles per milligram of protein per hour.

Metabolism of Citrate

Since the presence of fermentable carbohydrate may influence the extent of citrate utilization by lactobacilli (Campbell and Gunsalus 1944; Gibson and Abdel-Malek 1945; Fryer 1970), the metabolism of citrate was studied manometrically in whole-cell suspensions and cell-free extracts, at pH 5·5 and 7·0, both in the presence and absence of glucose. However, *L. acidophilus, L. bulgaricus, L. helveticus* and *L. lactis* used in this investigation did not utilize citrate under the conditions used (data not shown). Cell-free extracts (but not whole cells) of *L. plantarum* 343, utilized significant amounts of citrate in a manner similar to that described for *S. faecium* (Coventry *et al.* 1978):

$$\text{Citrate} + \tfrac{1}{2}O_2 \rightarrow 2 \text{ acetate} + 2CO_2 .$$

This presumably involved the hydrolysis of citrate to acetate and oxaloacetate, the decarboxylation of oxaloacetate to pyruvate, and the conversion of pyruvate to acetate via pyruvate oxidase. A small amount of diacetyl was also formed. The presence of glucose had no effect on the amount of citrate utilized.

L. casei 151 also utilized small amounts of citrate, under the conditions used, but the level of products formed was too low to determine the pathway of citrate metabolism.

Discussion

The results of this investigation, indicated that the strains of *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. delbrueckii*, *L. lactis*, and *L. plantarum* studied, all oxidized pyruvate via the pyruvate oxidase system. These organisms did not possess an active pyruvate dehydrogenase system and were unable to convert significant amounts of acetaldehyde to acetate suggesting that a yeast-type pyruvate decarboxylase was also not present.

Lloyd *et al.* (1978) showed that *L. bulgaricus* lacked both a pyruvate dehydrogenase system and a yeast-type pyruvate decarboxylase but possessed a pyruvate oxidase system which produced equimolar amounts of acetyl phosphate and acetate from pyruvate. However, in the present study this ratio of acetyl phosphate to acetate was not obtained, due to the degradation of acetyl phosphate both enzymically and non-enzymically. This difference could have been due to the differing methods of preparing the cell free extracts (sonic disruption versus French press). Dialysis of the cell-free extracts (to remove ADP) and the addition of sodium fluoride to the incubation mixture inhibited the enzymic hydrolysis of acetyl phosphate and allowed this compound to accumulate in the incubation mixture. Thus, acetyl phosphate was shown to be an intermediate in the conversion of pyruvate to acetate by all strains of *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. delbrueckii*, *L. lactis* and *L. plantarum* investigated. Campbell and Gunsalus (1944) and Fryer (1970) have shown that some lactobacilli were able to produce formic and acetic acids, which suggested a phosphoroclastic cleavage of pyruvate. This reaction does not produce CO_2 from pyruvate. The fact that the ratio of CO_2 evolved to pyruvate utilized was close to 1 : 1 for all the organisms studied suggested that the phosphoroclastic cleavage of pyruvate was not a major pathway of pyruvate utilization under the conditions used in this investigation.

In contrast to the lactobacillus species described above, none of the strains of *L. helveticus* tested were able to oxidize pyruvate to acetate under the same conditions.

The formation of lactate by *L. casei* 151 and *L. plantarum* 343 suggested a dismutation reaction in which reduced cofactors generated by the pyruvate oxidase enzyme were reoxidized by lactate dehydrogenase. Hager *et al.* (1954) have shown that the pyruvate oxidase from *L. delbrueckii* contained FAD and TPP and carried out a dismutation with the FAD-containing lactate dehydrogenase only when free riboflavin was added to the reaction mixture. While it is probable that the pyruvate oxidase of *L. casei* 151 and *L. plantarum* 343 also contained FAD, the lactate dehydrogenase of both organisms used only NADH as cofactor. In order for the dismutation reaction to occur there must be some mechanism whereby the reduced FAD, bound to the pyruvate oxidase, is converted to NADH. Such a mechanism has been shown to occur in the *E. coli* pyruvate dehydrogenase system (Massey 1963).

The absence of FAD from the incubation mixtures had no effect on pyruvate oxidase activity of dialysed cell-free extracts of *L. acidophilus*, *L. bulgaricus*, *L. delbrueckii* and *L. lactis* (data not shown) which suggested that FAD was tightly bound to the enzyme and not readily removed by dialysis. However, the addition of FAD was required for maximum enzyme activity in dialysed cell-free extracts of *L. plantarum* 343, which suggested that FAD was more loosely bound to the enzyme in this organism.

The ability of *L. casei* 151 and *L. plantarum* 343 to form acetoin suggested that the acetaldehyde-TPP complex, formed by the pyruvate oxidase system in these organisms, could condense with pyruvate to form α-acetolactate as described in other bacteria (Juni 1952*a*). Branen and Keenan (1972) have shown that *L. casei* has the enzymes to convert pyruvate to α-acetolactate and α-acetolactate to acetoin. *L. plantarum* 343 and *L. casei* 151 metabolized pyruvate to acetoin, lactate and acetate and *L. casei* 151 was also able

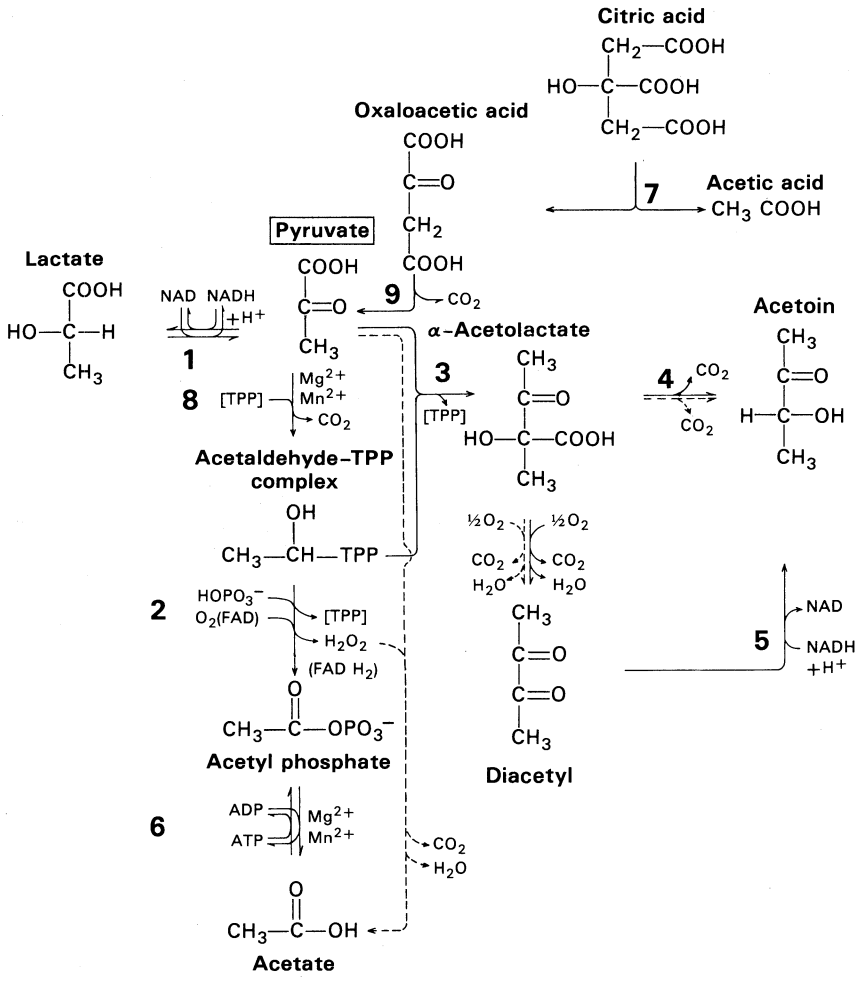

Fig. 1. Pathways of pyruvate and citrate metabolism in lactobacilli. Bold numerals indicate the enzyme systems involved as follows: **1**, lactate dehydrogenase; **2**, pyruvate oxidase; **3**, α-acetolactate synthase; **4**, α-acetolactase decarboxylase; **5**, acetoin dehydrogenase; **6**, acetate kinase; **7**, citrate (*pro*-3S)-lyase; **8**, pyruvate decarboxylase; **9**, oxaloacetate decarboxylase. Square brackets indicate enzyme-bound components. - - - - Indicates non-enzymic reaction.

to produce small amounts of diacetyl. It is possible that diacetyl could be produced by the condensation of the acetaldehyde-TPP complex with acetyl CoA described by Collins and Bruhn (1970). However, this is unlikely since treatment of the cell-free extract to remove CoA did not reduce diacetyl formation, nor did the addition of CoA increase its formation. In view of the end products detected, the metabolism of pyruvate by whole

cells of *L. casei* 151 appeared to proceed by the following pathways:

(i) Pyruvate dismutation:

$$2 \text{ Pyruvate} \rightarrow \text{lactate} + \text{acetate} + CO_2 \quad (1)$$

(ii) Pyruvate oxidase plus acetate kinase:

$$2 \text{ Pyruvate} + O_2 \rightarrow 2 \text{ acetate} + 2CO_2 \quad (2)$$

(iii) α-Acetolactate synthase (EC 4.1.3.18) plus decarboxylation of α-acetolactate:

$$2 \text{ Pyruvate} \rightarrow \text{acetoin} + 2CO_2 \quad (3)$$

(iv) α-Acetolactate synthase plus oxidative decarboxylation of α-acetolactate:

$$2 \text{ Pyruvate} + \tfrac{1}{2}O_2 \rightarrow \text{diacetyl} + 2CO_2 + H_2O \quad (4)$$

Reactions (1)–(4) accounted for approximately 38, 11, 48 and 3% respectively, of the pyruvate utilized by whole cells of *L. casei* 151. The same reactions appeared to be true for *L. plantarum* 343 except that no diacetyl was formed from pyruvate by this organism.

Earlier results (Rowatt 1951; Branen and Keenan 1971) had shown that free acetaldehyde had no effect on the production of acetoin and diacetyl from pyruvate in *L. casei* and *L. plantarum* and this was confirmed in the present study (data not shown). Also, none of the species tested could utilize acetaldehyde, either alone or in the presence of pyruvate, even when CoA was added to the reaction mixture.

Table 3. Presence (+) or absence (−) of enzyme systems involved in the metabolism of pyruvate and citrate in lactobacilli

Enzyme systems	*L. acido-philus*	*L. bulg-aricus*	*L. casei*	*L. delb-ruecki*	*L. helvet-icus*	*L. lactis*	*L. plant-arum*
1. Lactate dehydrogenase	+	+	+	+	+	+	+
FDP activation	−	−	+	−	−	−	−
2. Pyruvate oxidase	+	+	+	+	−	+	+
3. α-Acetolactate synthase	−	−	+	−	−	+	+
4. α-Acetolactate decarboxylase	−	−	+	−	−	−	+
5. Acetoin dehydrogenase	−	−	+	−	−	−	±
6. Acetate kinase	+	+	+	+	−	+	+
7. Citrate (*pro*-3S)-lyase	−	−	±	−	−	−	+
8. Pyruvate decarboxylase	−	−	+	−	−	−	+

A summary of the various metabolic pathways involved in the metabolism of pyruvate and citrate and their presence or absence in the lactobacilli examined in this investigation is shown in Fig. 1 and Table 3.

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References

Branen, A. L., and Keenan, T. W. (1970). Growth stimulation of *Lactobacillus casei* by sodium citrate. *J. Dairy Sci.* **53**, 593–8.

Branen, A. L., and Kennan, T. W. (1971). Diacetyl and acetoin production by *Lactobacillus casei*. *Appl. Microbiol.* **22**, 517–21.

Branen, A. L., and Keenan, T. W. (1972). Biosynthesis of α-acetolactate and its conversion to diacetyl and acetoin in cell-free extracts of *Lactobacillus casei*. *Can. J. Microbiol.* **18**, 479–85.

Campbell, J. J. R., and Gunsalus, I. C. (1942). Fermentation of citric acid by homofermentative lactic acid bacteria. *J. Bacteriol.* **44**, 721.

Campbell, J. J. R., and Gunsalus, I. C. (1944). Citric acid fermentation by streptococci and lactobacilli. *J. Bacteriol.* **48**, 71–6.

Chantrene, H., and Lipmann, F. (1950). Coenzyme A dependence and acetyl donor function of the pyruvate-formate exchange system. *J. Biol. Chem.* **187**, 757–67.

Christensen, M. D., Albury, M. N., and Pederson, C. S. (1958). Variation in the acetic acid–lactic acid ratio among the lactic acid bacteria. *Appl. Microbiol.* **6**, 316–18.

Christensen, M. D., and Pederson, C. S. (1958). Factors affecting diacetyl production by lactic acid bacteria. *Appl. Microbiol.* **6**, 319–22.

Chuang, L. F., and Collins, E. B. (1968). Biosynthesis of diacetyl in bacteria and yeast. *J. Bacteriol.* **95**, 2083–89.

Collins, E. B., and Bruhn, J. C. (1970). Roles of acetate and pyruvate in the metabolism of *Streptococcus diacetylactis*. *J. Bacteriol.* **103**, 541–6.

Coventry, M. J., Hillier, A. J., and Jago, G. R. (1978). The metabolism of pyruvate and citrate in the thermoduric cheese starter *Streptococcus faecium* (*Streptococcus durans*). *Aust. J. Dairy Technol.* **33**, 148–54.

Dirar, H., and Collins, E. B. (1972). End-products, fermentation balances and molar growth yields of homofermentative lactobacilli. *J. Gen. Microbiol.* **73**, 233–8.

Dirar, H., and Collins, E. B. (1973). Aerobic utilisation of low concentrations of galactose by *Lactobacillus plantarum*. *J. Gen. Microbiol.* **78**, 211–15.

Drinan, D. F., Tobin, S., and Cogan, T. M. (1976). Citric acid metabolism in hetero- and homofermentative lactic acid bacteria. *Appl. Environ. Microbiol.* **34**, 481–6.

French, C. S., and Milner, H. W. (1955). Disintegration of bacteria and small particles by high-pressure extrusion. In 'Methods in Enzymology'. (Eds S. P. Colowick, and N. O. Kaplan), Vol. 1. pp. 64–7. (Academic Press: New York.)

Fryer, T. F. (1970). Utilisation of citrate by lactobacilli isolated from dairy products. *J. Dairy Res.* **37**, 9–15.

Gardenas, I. L. B. de, Holgado, A. P. de Ruiz, and Oliver, G. (1980). The effect of pyruvate on the production of flavour compounds by *Lactobacillus casei* ATCC 7469. *Milchwissenschaft* **35**, 296–300.

Gibson, T., and Abdel-Malek, Y. (1945). The formation of carbon dioxide by lactic acid bacteria and *Bacillus licheniformis* and a cultural method of detecting the process. *J. Dairy Res.* **14**, 35–8.

Hager, L. P., Geller, D. M., and Lipmann, F. (1954). Flavoprotein-catalysed pyruvate oxidation in *Lactobacillus delbrueckii*. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **13**, 734–8.

Hunt, A. L., and Nossal, P. M. (1954). Investigations on glucose catalyse in bacterial metabolism. *Aust. J. Exp. Biol. Med. Sci.* **32**, 533–42.

Juni, E. (1952*a*). Mechanisms of formation of acetoin by bacteria. *J. Biol. Chem.* **195**, 715–26.

Juni, E. (1952*b*). Mechanisms of the formation of acetoin by yeast and mammalian tissues. *J. Biol. Chem.* **195**, 727–34.

Lipmann, F., and Tuttle, C. L. (1945). A specific micromethod for the determination of acyl phosphates. *J. Biol. Chem.* **159**, 21.

Lloyd, G. T., Hillier, A. J., Barlow, I., and Jago, G. R. (1978). The aerobic formation of acetate from pyruvate by *Lactobacillus bulgaricus*. *Aust. J. Biol. Sci.* **31**, 565–71.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. L. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–75.

Man, J. C. de, Rogosa, M., and Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.* **23**, 130–5.

Massey, V. (1963). In 'The Enzymes'. (Eds P. D. Boyer, H. Lardy, and K. Myrback.) 2nd Edn. Vol. 7. pp. 275–306. (Academic Press: New York and London.)

Noll, F. (1974). In 'Methods of Enzymic Analysis'. (Ed. H. V. Bergmeyer.) Vol. 3. pp. 1475–9. (Academic Press: New York.)

Nossal, P. M. (1952). The effect of glucose and potassium on the metabolism of pyruvate in *Lactobacillus arabinosus*. *Biochem. J.* **50**, 591–5.

Rowatt, E. (1951). The metabolism of pyruvate by *Lactobacillus plantarum*. *Biochem. J.* **49**, 453–62.

Stadtman, E. R. (1950). Coenzyme A-dependent transacetylation and transphosphorylation. *Fed. Proc.* **9**, 233.

Terzaghi, B. E., and Sandine, W. E. (1975). Improved medium for lactic streptococci and the bacteriophages. *Appl. Microbiol.* **29**, 807–13.

Thomas, M. P., Broome, M. C., Hillier, A. J., and Jago, G. R. (1979). A comparative study of the formation of acetate from pyruvate in group N streptococci. *Aust. J. Dairy Technol.* **34**, 97–100.
Umbreit, W. W., Burnis, R. H., and Stauffer, J. F. (1957). 'Manometric Techniques'. 3rd Edn. (Burgess: Minneapolis.)
Vanderheiden, G. J., Fairchild, A. C., and Jago, G. R. (1970). Construction of a laboratory press for use with the French pressure cell. *Appl. Microbiol.* **19**, 875.
Von Korff, R. W. (1969). In 'Methods in Enzymology'. (Ed. J. M. Lowenstein.) Vol. 13. p. 521. (Academic Press: New York and London.)

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