Pyruvate Metabolism and the Phosphorylation State of Isocitrate Dehydrogenase in Escherichia coli

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During growth of Escherichia coli on acetate, isocitrate dehydrogenase (ICDH) is partially inactivated by phosphorylation and is thus rendered rate-limiting in the Krebs cycle so that the intracellular concentration of isocitrate rises which, in turn, permits an increased flux of carbon through the anaplerotic sequence of the glyoxylate bypass. A large number of metabolites stimulate ICDH phosphatase and inhibit ICDH kinase in the wild-type (E. coli ML308) and thus regulate the utilization of isocitrate by the two competing enzymes, ICDH and isocitrate lyase. Addition of pyruvate to acetate grown cultures triggers a rapid dephosphorylation and threefold activation of ICDH, both in the wild-type (ML308) and in mutants lacking pyruvate dehydrogenase (ML308/Pdh−), PEP synthase (ML308/Pps−) or both enzymes (ML308/Pdh−Pps−). Pyruvate stimulates the growth on acetate of those strains with an active PEP synthase but inhibits the growth of those strains that lack this enzyme. When pyruvate is exhausted, ICDH is again inactivated and the growth rate reverts to that characteristic of growth on acetate. Because pyruvate stimulates dephosphorylation of ICDH in strains with differing capabilities for pyruvate metabolism, it seems likely that pyruvate itself is a sufficient signal to activate the dephosphorylation mechanism, but this does not discount the importance of other signals under other circumstances.

INTRODUCTION

The growth of Escherichia coli on acetate requires the operation of the glyoxylate bypass (Fig. 1) in which isocitrate lyase (ICL; EC 4.1.3.1) must compete with isocitrate dehydrogenase (ICDH; EC 1.1.1.42) for isocitrate (Ashworth & Kornberg, 1963, 1964; Kornberg, 1966). However, ICDH has a much higher affinity for isocitrate than does ICL (Walsh & Koshland, 1984; C. MacKintosh & H. G. Nimmo, unpublished results). Inactivation of a large part of the ICDH allows intracellular isocitrate to rise to a concentration at which ICL can operate (El-Mansi et al., 1985), and as soon as the glyoxylate bypass is not required, ICDH is rapidly reactivated (Holms & Bennett, 1971; Bennett & Holms, 1975). Inactivation is by phosphorylation and reactivation is by dephosphorylation (Garnak & Reeves, 1979a, b; Borthwick et al., 1984b), catalysed by a single bifunctional enzyme, ICDH kinase/phosphatase (LaPorte & Koshland, 1982; Nimmo et al., 1984) which, in turn, is regulated by a variety of metabolites (LaPorte & Koshland, 1983; Nimmo & Nimmo, 1984). Pyruvate is a potent effector in vitro (Nimmo & Nimmo, 1984). However, it is difficult to assess whether pyruvate itself, or metabolites derived from it, are the important effectors in vivo. The purpose of this paper is firstly to describe the effect of pyruvate on ICDH activation in mutants deficient in either pyruvate dehydrogenase (ML308/Pdh−) or phosphoenolpyruvate (PEP) synthase (ML308/Pps−) or both (ML308/Pdh−Pps−) (because of these lesions the mutants may have some difficulty in generating effectors of the ICDH kinase/phosphatase from pyruvate), and secondly, to show that the co-utilization of pyruvate and acetate is related to growth rate and efficiency of substrate conversion to biomass.

Abbreviations: ICDH, isocitrate dehydrogenase; ICL, isocitrate lyase; PEP, phosphoenolpyruvate.
Fig. 1. Metabolic interrelations among the key enzymes of acetate metabolism and the central pathways. The glyoxylate bypass enzymes are indicated by heavy arrows. The key enzymes are as follows: (1) acetate activating enzymes (two systems are now known, i.e. acetyl-CoA synthetase and acetokinase and phosphotransacetylase); (2) isocitrate lyase; (3) malate synthase; (4) PEP carboxykinase; (5) pyruvate kinase.

METHODS

Chemicals. Purified [U-14C]pyruvic acid, sodium salt, was obtained from Amersham. [14C]Toluene, PPO and POPOP were from Packard. Silicagel 60 G (Kieselgel 60G) was from Merck. Protosol was from New England Nuclear. All other chemicals were as described by Borthwick et al. (1984a) and Holms & Robertson (1974), or they were of Analar grade from BDH.

Bacterial strains. The organisms used were Escherichia coli ML308 (ATCC 15224) which, apart from the constitutive expression of the lactose operon enzymes, is regarded as wild-type, and three mutants derived from it: a pyruvate dehydrogenase-less strain (ML308/Pdh-); a phosphoenolpyruvate synthase-less strain (ML308/Pps-), and a strain lacking both enzymes (ML308/Pdh-Pps-). The ML308/Pdh- strain and the ML308/Pps- strain were derived directly from the parent strain whereas the double mutant ML308/Pdh-Pps- was derived from the ML308/Pps- strain (Holms & Robertson, 1974).

Characterization of the mutants. Lyophilized samples of the strains were resuscitated in nutrient broth and shown to have the growth characteristics (Table 1) typical of their genotype (Holms & Robertson, 1974) as well as the ability to synthesize β-galactosidase constitutively, which is characteristic of the parent strain (Cohn & Monod, 1951). The mutants cannot grow on pyruvate (Tab. 1) as sole carbon source because of their inability to generate acetyl-CoA or PEP or both. Neither of the mutants lacking pyruvate dehydrogenase activity was able to grow on succinate as they cannot generate acetyl-CoA from pyruvate.

Growth conditions. The parent strain as well as the three derivatives were maintained as previously described by Holms & Robertson (1974), and were grown in batch cultures (800 ml) aerobically at 37 °C (Harvey et al., 1968) in a simple defined medium containing 40 mM-KH₂PO₄ (adjusted to pH 7 with NaOH), 10 mM-(NH₄)₂SO₄, 2 mM-MgSO₄.7H₂O, 10 μM-FeSO₄.7H₂O (adjusted to pH 2 with HCl), supplemented with acetate at a final concentration of 40 mM. Acetate, magnesium and iron salts were autoclaved as separate solutions and the complete growth medium was made up just before inoculation. The final pH of the medium was 7.0. In all experiments inocula were prepared by passaging the appropriate strain three times on acetate as sole source of carbon. Growth was followed by measuring the OD₆₅₀ in a single beam flow cell Pye Unicam SP 30 spectrophotometer.

Assay of enzyme activities. β-Galactosidase and ICDH were assayed as previously described by Holms & Robertson (1974) and Bennett & Holms (1975), respectively, in a Pye Unicam SP8-100 spectrophotometer.
Table 1. Growth and β-galactosidase activity of strains under investigation

Each value is the mean of three experiments; the range of results (in parentheses) is expressed as a percentage of the mean.

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Pdh⁺Pps⁺</th>
<th>Pdh⁻Pps⁺</th>
<th>Pdh⁺Pps⁻</th>
<th>Pdh⁻Pps⁻</th>
</tr>
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<tbody>
<tr>
<td>Acetate</td>
<td>8.72 (± 2.5%)</td>
<td>7.81 (± 1.2%)</td>
<td>8.12 (± 2.1%)</td>
<td>2.94 (± 4.6%)</td>
</tr>
<tr>
<td>M.g.t.†</td>
<td>120 (± 1.7%)</td>
<td>190 (± 1.6%)</td>
<td>184 (± 1.1%)</td>
<td>188 (± 1.1%)</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>3.08 (± 4.4%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M.g.t.</td>
<td>58 (± 1.7%)</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>Succinate</td>
<td>9.86 (± 2.6%)</td>
<td>-</td>
<td>8.52 (± 2.2%)</td>
<td>-</td>
</tr>
<tr>
<td>M.g.t.</td>
<td>64 (± 4.7%)</td>
<td>NG</td>
<td>76 (± 3.3%)</td>
<td>NG</td>
</tr>
</tbody>
</table>

NG, No growth.
* β-Galactosidase activity [µmol o-nitrophenol released (mg protein)⁻¹ min⁻¹ at 27 °C].
† Mean generation time (min).

Acetate and pyruvate estimations. Portions (4 ml) of growing culture were added to 2 g ice in a test-tube (125 × 16 mm) and mixed until the ice was completely melted. Bacteria were then removed by centrifugation at 20000 g for 10 min at 4 °C and the supernatant was decanted and stored at −15 °C until required. Frozen samples were thawed and any traces of pellet were once again removed by centrifugation. Where appropriate, dilutions of samples and standards were made using carbon source-free medium (diluted 1:3 in water). Acetate and pyruvate were determined enzymically according to the methods of Bergmeyer & Möllering (1974) and Bücher et al. (1963), respectively.

¹⁴CO₂ absorption. Evolved ¹⁴CO₂ was trapped by bubbling the effluent gas through 10 ml Protosol (quaternary ammonium hydroxide) in an appropriate scintillation glass vial connected to a Vigreux column to allow maximum efficiency of absorption. The ¹⁴CO₂ was collected over 20 min periods and after each collection the column was washed with 5 ml Protosol to give a final volume of 15 ml for each sample.

Culture filtrates and solubilization of cell pellets. Portions (6 ml) were withdrawn from the growing culture at intervals of 20 min, centrifuged at 20000 g for 10 min at 4 °C, and the supernatant used for radioactivity determination in a toluene based scintillation fluid containing PPO and POPOP (Woeller, 1961). The cell pellet was resuspended in medium free of carbon source and harvested by centrifugation at 25000 g. The pellet was decolourized by heating (55 °C, 10 min) with 100 µl 10 volume HzO₂, then cooled, mixed with 10 ml scintillant, and counted.

Counting efficiency. Samples were counted in a mark III liquid scintillation system model 6880 (Searle). The efficiency of counting in the presence of various amounts of aqueous phases was determined using chloroform as the polar quenching agent and [¹⁴C]toluene as the radioactive standard.

High performance liquid chromatography. This was done at room temperature on an Aminex HPX-87H organic acid column (Bio-Rad). The column was eluted with 5 mM-H₂SO₄ at a flow rate of 1 ml min⁻¹. The effluent was monitored at 215 nm to detect pyruvate, acetate and other intermediates. Chromatography of authentic compounds was also done to verify the identity of the intermediates resolved.

Preparation of 2,4-dinitrophenylhydrazone derivatives for thin layer chromatography (TLC). Samples of culture filtrates, containing approximately 10 µmol product, and aqueous solutions containing 10 µmol authentic acetaldehyde, as a standard, were treated with excess 0.1% (w/v) 2,4-dinitrophenyldihydrazine in 2 M-HCl and incubated at 30 °C for 30 min (Friedemann & Haugen, 1943). The water insoluble hydrazone derivatives of non-acidic aldehydes or ketones were then separated by centrifugation at 25000 g for 10 min. The pellets were washed once with 2 M-HCl to remove unreacted 2,4-dinitrophenylhydrazine, and then washed twice with distilled H₂O to remove the acid. The precipitates were dissolved in 2-3 ml ethyl acetate and passed through a column of anhydrous Na₂SO₄ to remove H₂O. After the column had been washed with 3 ml ethyl acetate, to ensure full recovery of phenylhydrazone derivatives, ethyl acetate was evaporated under a stream of nitrogen at 45 °C, and the residue was dissolved in 0.2 ml ethyl acetate and used for TLC.

TLC. This was done on silicagel G plates (0.25 mm) which were originally prepared by coating five plates with a jump-free slurry of 30 g kieselgel 60 G in 60 ml glass distilled H₂O. The plates were air-dried and activated at 110 °C for 60 min, and then stored in a desiccator until required. Samples were applied and chromatograms were developed in rectangular glass tanks containing chloroform/acetic acid (100:7, v/v), and lined with filter paper to ensure saturation of the atmosphere with the solvent (Seakins et al., 1976). Dinitrophenylhydrazone derivatives were detected under UV light as dark spots on the chromatogram.
RESULTS AND DISCUSSION

Effectors of ICDH activation

When any of the mutants or the parent strain are growing on acetate the specific activity of ICDH is kept at the low level (Figs 2b, 3b and 4b) characteristic of inactivation by phosphorylation (Bennett & Holms, 1975; Garnak & Reeves, 1979a, b; Borthwick et al., 1984b). In all the strains, addition of pyruvate triggers an immediate activation (dephosphorylation) of ICDH which persists as long as pyruvate remains in the medium, but reversion of ICDH to the activity characteristic of growth on acetate occurs as soon as pyruvate is exhausted (Figs 2b, 3b and 4b). Data for the Pdh−Pps+ mutant, which behaves similarly to the parent, are not shown.

Pyruvate is only one of a family of compounds which are effectors of the regulatory enzyme ICDH-kinase/phosphatase (LaPorte & Koshland, 1983; Nimmo & Nimmo, 1984) which both phosphorylates and dephosphorylates ICDH (LaPorte & Koshland, 1982; Nimmo et al., 1984). In principle, any number or combination of these effectors could be generated on pyruvate addition and thus signal the activation of ICDH. The effectors could include pyruvate itself or any other compound derived from either pyruvate or acetate, both of which are metabolized during the period of high ICDH activity (Figs 2b, 3b and 4b), although the mutants must differ from the parent strain (and each other) in their ability to convert pyruvate into new compounds. The changes in intracellular concentrations of effectors could result from four different variations of metabolic flux (or some combination of them).

1. Entry of pyruvate raises its intracellular concentration to a level where it becomes the major signal for ICDH activation.

2. Exogenous pyruvate satisfies the biosynthetic requirement for pyruvate and thus lowers the demand on the glyoxylate bypass, which allows this pathway to channel a greater flux of carbon to the biosynthesis of other effectors.

3. The supply of pyruvate is sufficient to sustain biosynthesis of all the metabolites, including the signals for ICDH activation, and the glyoxylate bypass becomes redundant. This was the original hypothesis for the effect of pyruvate (Bennett & Holms, 1975), and would also involve a much diminished flux through the bypass because of inhibition of ICL by PEP (Ashworth & Kornberg, 1963, 1964).

4. The changes in carbon flux through the competing pathways alters the energy charge and the NADPH/NADP+ ratio rather than the concentrations of metabolites used as precursors of carbon for biosynthesis. This would happen if the flux of pyruvate into the cell diverted the supply of energy from the mechanisms for converting acetate to acetyl-CoA (Matin & Konings, 1973; Brown et al., 1977), and would be particularly effective in those mutants which lacked the route from pyruvate to acetyl-CoA.

Clearly the intracellular concentration of pyruvate must be important either in itself (1), or because it is required to sustain the flow of carbon hypothesized in (2)–(4). However, the ability of the various mutants to satisfy biosynthesis is impaired in a variety of ways. It follows that an elevated intracellular concentration of pyruvate is basic to all reasonable hypotheses for the mechanism of ICDH activation. Although other signals such as isocitrate do rise (El-Mansy et al., 1985), we conclude that pyruvate itself is a major signal in these strains under these circumstances (1 above). Unfortunately the high extracellular concentration of pyruvate makes it impossible to determine its intracellular concentration.

Effects on growth and metabolic activity

Compared with the parent strain, the mutants take longer to metabolize the added pyruvate; the parent (Pdh+Pps+) takes 35 min while the Pdh+Pps− and Pdh−Pps+ mutants require about 80 min and the Pdh−Pps− mutant needs about 100 min. Pyruvate slightly stimulates growth in both strains with active PEP synthase (Fig. 2a and data not shown), presumably by increased flux of carbon to biosynthetic precursors (2 and 3 above). Many of these are effectors of ICDH activation (Nimmo & Nimmo, 1984) and thus reinforce the activation by pyruvate itself. In the two strains lacking PEP synthase growth is slowed by pyruvate (Figs 3a and 4a). This could be due to a fall in the rate of energy supply (4 above). However, acetate is still metabolized (Figs 3b and 4b), and it is likely that the Krebs cycle still operates because of the
Control of E. coli isocitrate dehydrogenase

![Graph showing the effect of pyruvate on growth and ICDH activity.](image)

**Fig. 2.** Effect of pyruvate (0.5 mM) on (a) growth and (b) ICDH activity and substrate utilization by E. coli ML308 growing aerobically on acetate (40 mM) in a batch culture at 37 °C (Hamilton & Holmes, 1970). At intervals, samples of culture were assayed for optical density (dry wt: □, control; ■, pyruvate addition), and ICDH activity (●) was assayed in cells broken by ultrasonic irradiation (Bennett & Holmes, 1975). Acetate (▲) and pyruvate (○) were measured enzymically in the supernatant. Pyruvate was added at the time indicated by the arrow.

High activity of ICDH (Figs 3b and 4b) in both these strains. This would apply particularly to the Pdh+Pps- strain which can supply acetyl-CoA directly to the Krebs cycle from pyruvate. Therefore, in these mutants, the slower growth rate is presumably due to a lowered intracellular content of biosynthetic precursors. This could reasonably be due to the inability of these strains to channel pyruvate to biosynthetic functions, and to the higher ICDH activity favouring energy production by the Krebs cycle at the expense of ICL activity and biosynthesis by the glyoxylate bypass. It follows again that, in these strains, pyruvate itself is the important effector of ICDH activation.

It is difficult to assess the effects of ICDH activation on metabolic systems when both rate and efficiency of conversion of substrate to biomass are affected. It is therefore desirable to derive from the primary data the relationship of pyruvate and acetate utilization to biomass formation (growth). For all strains, these are directly proportional to each other (Figs 2b, 3b and 4b), and the slopes of the lines relating substrate concentration to bacterial dry weight can be expressed as mmol carbon source utilized per g dry weight new biomass formed (Table 2). These figures are the reciprocal of growth yields and can be converted into rates [mmol substrate used (g dry wt biomass)⁻¹ h⁻¹] by reference to the rate (μ) at which biomass is formed (Table 2).

For growth on acetate alone, the mutants convert this carbon source to new biomass with the same efficiency as, but at a slower rate than, the parent strain (Table 2); this is related to the slower growth rate observed (Figs 2a, 3a and 4a). It is difficult to see how loss of either or both of pyruvate dehydrogenase and PEP synthase diminishes the rate of metabolism and growth on acetate. Although PEP carboxykinase is essential for growth on acetate (Hsie & Rickenberg, 1966; Sanwal, 1970), neither pyruvate dehydrogenase nor PEP synthase is thought to be.
Effect of pyruvate (0.5 mM) on (a) growth and (b) ICDH activity and substrate utilization by E. coli ML308/Pps-. Growth conditions and symbols are as described in the legend to Fig. 2.

Table 2. Utilization of substrates

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Acetate</th>
<th>Acetate and pyruvate</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Rate</td>
<td>Rate</td>
</tr>
<tr>
<td>Strain</td>
<td>utilized</td>
<td>utilized</td>
</tr>
<tr>
<td></td>
<td>(mmol g⁻¹)</td>
<td>(mmol g⁻¹ h⁻¹)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(mmol g⁻¹)</td>
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<td>Pdh⁺ Pps⁺</td>
<td>62</td>
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<td>13.4</td>
</tr>
<tr>
<td>Pdh⁺ Pps⁻</td>
<td>60</td>
<td>13.6</td>
</tr>
<tr>
<td>Pdh⁻ Pps⁻</td>
<td>59</td>
<td>12.9</td>
</tr>
</tbody>
</table>

However, this does not mean to say that they have no function during growth on acetate. For example, malic enzyme and PEP synthase could supplement the provision of PEP by PEP carboxykinase. Loss of the two enzymes is known to affect at least one other apparently unrelated function in that constitutive β-galactosidase synthesis (characteristic of all these strains) is strongly repressed (Table 1) in the Pdh⁻ Pps⁻ mutant but not in the others (Holms & Robertson, 1974).

Addition of pyruvate to acetate-grown cultures affects the strains in various ways, the only feature common to all being activation (dephosphorylation) of ICDH. In the parent strain (Pdh⁺ Pps⁺) pyruvate slightly stimulates the growth rate (Fig. 2a) and diminishes the amount and rate of acetate utilization, but the total carbon utilization is increased (Table 2). Presumably
Control of E. coli isocitrate dehydrogenase

Fig. 4. Effect of pyruvate (0.5 mM) on (a) growth and (b) ICDH activity and substrate utilization by E. coli ML308/Pdh-Pps-. Growth conditions and symbols are as described in the legend to Fig. 2.

the generation of intermediates from pyruvate by PEP synthase lessens the demands made on the glyoxylate bypass. This allows the increased activity of ICDH and the Krebs cycle to sustain the faster growth rate but the overall efficiency of carbon conversion to biomass is lower than that during growth on acetate. The most efficient mutant is the Pdh-Pps+ strain. Growth is stimulated slightly by pyruvate, again presumably, by use of PEP synthase and the more active ICDH, but the fall in demand for acetate is exactly balanced by the amount of pyruvate used so that the overall efficiency of carbon utilization is maintained (Table 2). The effect of pyruvate on acetate-grown cultures of the Pdh+Pps- mutant is most dramatic, with a fall in growth rate (μ) from 0.22 to 0.07 h⁻¹ which persists as long as pyruvate remains in the system (Fig. 3a). This presumably is a consequence of the activation of ICDH which competes with ICL thus lowering the supply of intermediates which cannot otherwise be generated because the strain is Pps-. This mutant is also the least efficient at carbon conversion in that acetate utilization is not spared, and all the pyruvate metabolized, or its equivalent, is wasted (Table 2). While its overall rate of metabolism is the lowest of all the mutants, pyruvate is metabolized at 69% of the rate at which acetate is metabolized (Table 2). The double mutant (Pdh-Pps-) suffers less when pyruvate is added, because ICDH is not so greatly activated. Growth rate falls from 0.22 to 0.14 h⁻¹, presumably for reasons similar to those for the Pdh+Pps- mutant, but the overall efficiency is less affected. While no acetate is spared, relatively less pyruvate is used (Table 2) and the rate of pyruvate utilization is only 45% of the rate of acetate utilization (Table 2).

Some general deductions can be made from these experiments. In order to take advantage of the increase in ICDH activity caused by pyruvate, a source of intermediates alternative to the glyoxylate bypass must be available and is provided by PEP synthase. Thus, loss of PEP synthase is a great disadvantage. In contrast, pyruvate dehydrogenase activity does not help in these situations and its activity decreases the efficiency of carbon conversion to biomass. Thus, the mutant most fitted to take advantage of pyruvate addition to acetate-grown cultures is the Pdh- Pps+ strain, and that least able to cope is the Pdh+Pps- strain (Table 2; Fig. 3).
The problem remains as to how the Pdh⁻Pps⁻ strain metabolizes pyruvate (Fig. 4b). None of the potential products of pyruvate metabolism (Fig. 5) could be found in acetate/pyruvate cultures of the Pdh⁻Pps⁻ strain. However, it would not be possible to detect a maximum of 0.5 mM-acetate derived from pyruvate in a medium containing 34 mM-acetate falling to 32.5 mM as the pyruvate was utilized (Fig. 4b). Accordingly, this experiment was repeated using [U-¹⁴C]pyruvate, which enables metabolic products to be traced. In particular, because the pyruvate was uniformly labelled, the ¹⁴C content of the acetate in the system permits the amount of pyruvate converted to acetate to be measured. The period of the pyruvate effect was prolonged (from 100 to 160 min) by increasing the pyruvate added (from 0.5 to 1.0 mM) but otherwise results were the same as in Fig. 4. [U-¹⁴C]Pyruvate is converted by the Pdh⁻Pps⁻ mutant to equimolar amounts of [U-¹⁴C]acetate and ¹⁴C₀₂ (data not shown). As acetaldehyde co-elutes with acetate in our HPLC system it is possible that some of the aldehyde is included with the acid but, from the sensitivity of our assay for the acetaldehyde by 2,4-dinitrophenylhydrazine formation, this must be less than 3% of the total. The mechanism by which this mutant converts pyruvate to acetate is not germane to this paper but it probably uses pyruvate oxidase, which is known to be derepressed in strains of E. coli with nonfunctional pyruvate dehydrogenase (Gounaris & Hager, 1961; Ingledew & Poole, 1984). As pyruvate oxidase donates electrons directly to an ATP-generating system (Shaw-Goldstein et al., 1978; Ingledew & Poole, 1984) it is likely that the Pdh⁻Pps⁻ mutant has an adequate energy supply. It follows that the lower growth rate reflects a diminished supply of a biosynthetic intermediate, which again underlines the importance of pyruvate itself as the primary signal for ICDH activation in this mutant under these circumstances.
In conclusion, pyruvate itself is a signal sufficient to trigger activation of ICDH. The strains best able to take advantage of this situation are those which retain PEP synthase and are thus able to generate intermediates from both pyruvate and acetyl-CoA. In contrast, those strains which retain pyruvate dehydrogenase only add to the supply of the primary metabolite of the acetate phenotype (acetyl-CoA) and are at a disadvantage.

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