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The fermentation pathways of *Escherichia coli*

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1. SUMMARY

Under anaerobic conditions and in the absence of alternative electron acceptors *Escherichia coli* converts sugars to a mixture of products by fermentation. The major soluble products are acetate, ethanol, lactate and formate with smaller amounts of succinate. In addition the gaseous products hydrogen and carbon dioxide are produced in substantial amounts. The pathway generating fermentation products is branched and the flow down each branch is varied in response both to the pH of the culture medium and the nature of the fermentation substrate. In particular, the ratio of the various fermentation products is manipulated in order to balance the number of reducing equivalents generated during glycolytic breakdown of the substrate. The enzymes and corresponding genes involved in these fermentation pathways are described. The regulatory responses of these genes and enzymes are known but the details of the underlying regulatory mechanisms are still obscure.

2. INTRODUCTION

The natural habitat of *Escherichia coli* is the large intestine of humans and other animals, from which *E. coli* emerged to colonize the scientific laboratory about half a century ago. The precise nature of the nutrients used by *E. coli* in the wild remains obscure [1]. Nonetheless, since growth normally occurs in the absence of oxygen, it is clear that energy must be supplied either by anaerobic respiration coupled to electron acceptors such as nitrate, trimethylamine oxide or fumarate, or by fermentation [2]. It is the latter alternative which is the subject of this review. *Escherichia coli* is a facultative anaerobe and as such can grow both aerobically or anaerobically by using sugars, such as glucose, as sole carbon and energy source [2,3]. Irrespective of the presence or absence of oxygen, glucose is transported into the cell by the phosphotransferase system and then catabolized to pyruvate [2]. It is in the further metabolism of pyruvate that differences between aerobic and anaerobic conditions take effect. In air, NADH generated both during glycolysis and by the Krebs cycle and associated reactions is reoxidized by operation of the respiratory chain [4]. During fermentation neither the respiratory chains linked to oxygen nor those linked to alternative electron acceptors are functional. The Krebs cycle [5,6] and pyruvate dehydrogenase [7,8] reactions which generate NADH in large amounts are inoperative under anaerobic conditions. However, NADH produced by glycolysis must be reoxidized

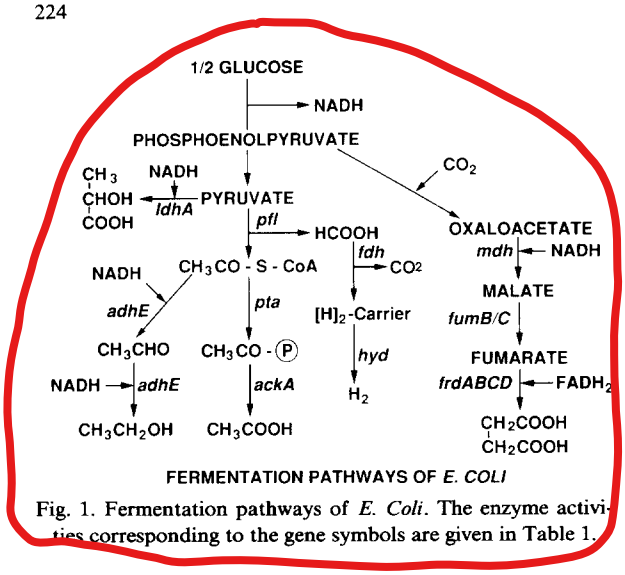

Fig. 1. Fermentation pathways of *E. Coli*. The enzyme activities corresponding to the gene symbols are given in Table 1.

to NAD^+ so that the glycolytic sequence can proceed [Figs. 1 and 2]. The key issue in fermentation is thus the recycling of NADH by conversion of pyruvate to fermentation products (Fig. 1 and Table 1). The genes coding for the enzymes of fermentation are listed in Table 1 and their positions on the E. coli chromosome are shown in Fig. 3.

During aerobic growth, facultative anaerobes such as *E. coli* metabolize pyruvate to acetyl CoA by means of the pyruvate dehydrogenase (PDH) complex:

$$CH_3COCOOH + CoASH + NAD^+ \rightarrow CH_3CO\text{-}SCoA + CO_2 + NADH + H^+$$

Anaerobically synthesis of the PDH complex is largely repressed and residual activity is presumably inhibited in vivo possibly by NADH [9]. This step is replaced by pyruvate formate lyase (PFL) [2,10]:

$$CH_3COCOOH + CoASH \rightarrow CH_3CO\text{-}SCoA + HCOOH$$

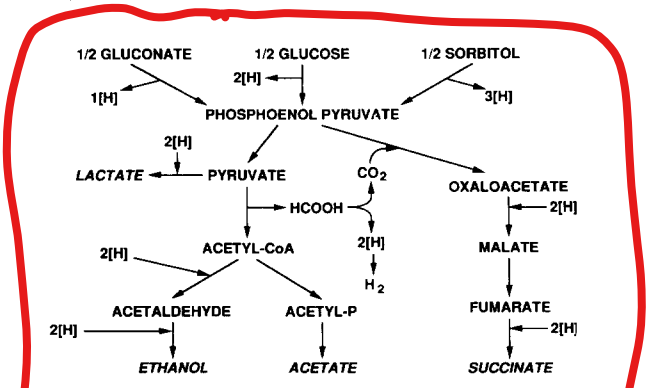

Fig. 2. Redox balance. The flow of reducing equivalents, [H], is shown for three possible starting substrates.

Table 1

Genes and enzymes

Gene	Map position	Function
acd	63	activity of ACDH
ackA	50	acetate kinase
ackB	39	acetate kinase
acs	50	acetyl-CoA synthetase
adhB	18	level of ADH/ACDH
adhC	27	probably promoter of *adhE*
adhE	27	Structural gene ADH/ACDH
adhR	72	negative regulation of *adhE*
ana	27	regulation of *adhE*
fdhF	92	formate dehydrogenase of formate hydrogen lyase complex
fnr	29	activator for anaerobic respiration
frdABCD	94	fumarate reductase
fumB	93	fumarase B (anaerobic)
fumC	36	fumarase C
ldhA	30	lactate dehydrogenase
ldhB	?	lactate dehydrogenase
mdh	70	malate dehydrogenase
pfl	20	pyruvate formate lyase
ppc	89	phosphoenolpyruvate carboxylase
pta	50	phosphotransacetylase

The map locations are taken from Bachmann [97].

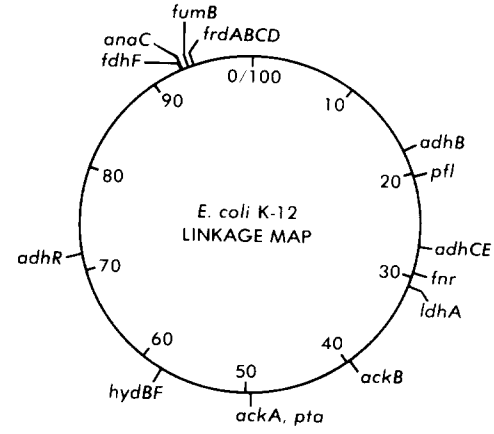

Fig. 3. Linkage map of *E. coli* K-12. Genes involved in fermentation are shown. For details for each gene see Table 1.

Acetyl-CoA is still produced but CO_2 and reduced NADH are replaced by the release of formic acid [Fig. 1]. This avoids the necessity of reoxidizing the NADH which would be produced by the PDH complex.

During conditions of high pyruvate accumulation or at low pH, pyruvate may be converted to lactate by the fermentative lactate dehydrogenase (LDH) [11,12]:

$$CH_3COCOOH + NADH + H^+ \rightarrow CH_3CHOHCOOH + NAD^+$$

A final alternative is the conversion of pyruvate or phosphoenolpyruvate (PEP) to a C4 intermediate of the Krebs cycle by the incorporation of CO_2 [13]. The phosphoenolpyruvate carboxylase (PPC) converts PEP to oxaloacetate [14] whereas the two malic enzymes (one linked to NAD, the other to NADP) can interconvert pyruvate and malate [2,14]. Whether the malic enzymes contribute significantly to synthesis of malate under anaerobic conditions is unknown. Both oxaloacetate and malate may be used as biosynthetic precursors or may be further reduced to succinic acid via fumarate [2,3]. Although CO_2 is often said to originate in the 'atmosphere' it is obvious that in a liquid bacterial culture the CO_2 is of metabolic origin. For anaerobic cultures this means CO_2 released from formate by the formate hydrogen lyase system [2] since under these conditions neither the PDH complex nor the Krebs cycle are operative. Thus the excreted succinate consists of three carbons from one pyruvate molecule and a fourth carbon originating from the carboxyl group of another pyruvate molecule [Fig. 2]. Succinic acid typically comprises around 5–10 mol % of the non-gaseous fermentation products [3,15–17]. Since only about 10% of the carbon source is assimilated during fermentative growth, decarboxylation reactions such as gluconate-6-phosphate dehydrogenase and isocitrate dehydrogenase would release only small amounts of CO_2. However, at neutral to alkaline pH, the formate hydrogen lyase system is poorly active and such minor pathways would become proportionately more significant contributors to succinate synthesis.

3. REDOX BALANCE

As remarked above, the key issue in fermentation is the recycling of reduced NADH to regenerate the oxidized form, NAD^+, so that glycolysis may continue. Since the amount of NADH to be recycled varies with the nature of the substrate, so must the composition of the mixture of fermentation products [Table 2 and Fig. 2]. Hexoses such as glucose or fructose produce 2 NADH per C6 upon conversion to pyruvate whereas hexitols (e.g. sorbitol or mannitol) produce 3 NADH per C6. Conversely the more oxidized sugar derivatives such as hexonic and hexuronic acids yield less than 2 NADH per C6. To achieve a proper fermentation balance it is necessary to match the NADH produced with the NADH consumed by excretion of fermentation products. In practice *E. coli* uses a mixture of ethanol, lactate and acetate, all of which consume different amounts of [H] per C6 [Table 2]. By varying the proportions of these it is possible to match the substrate so achieving redox balance [Fig. 2] [18]. Table 2 lists the reducing equivalents produced ('+') and consumed ('−') by various substrates and fermentation products.

Table 2

Reducing equivalents

Molecule	Formula	[H] per C6 *
Substrates		
Sorbitol	$C_6H_{14}O_6$	+6
Glucose	$C_6H_{12}O_6$	+4
Gluconate	$C_6H_{12}O_7$	+2
Glucuronate	$C_6H_{10}O_7$	0
Products		
Ethanol	CH_3CH_2OH	−8
Lactate	$CH_3CHOHCOOH$	−4
Acetate	CH_3COOH	0
Succinate (C3 + C1) +	CH_2COOH | CH_2COOH	−8
Succinate (C2 + C2) +		+2

* Sign convention '+' = produced during glycolysis; '−' = consumed during fermentation. + The two entries for succinate refer respectively to succinate formed from PEP plus CO_2 (C3 + C1 succinate) and from the glyoxylate cycle (C2 + C2 succinate). These two pathways consume different numbers of reducing equivalents.

Since it is possible to balance most fermentations by mixing ethanol and acetate in appropriate proportions, the production of lactate is unnecessary. As expected, *ldhA* and *ldhB* mutants, both of which lack LDH, show no anaerobic growth defects [19,20]. Production of ethanol is necessary only for substrates more reduced than glucuronate. Hence, *adhE* mutants, which lack alcohol dehydrogenase, cannot grow anaerobically on sorbitol, glucose or gluconate but will ferment glucuronate [21,22]. It might be thought that an *adhE* mutant could ferment gluconate by mixing acetate with lactate. However, this does not happen and it appears that the production of lactate is a response primarily to an acid pH [10,18]. Mutants defective in phosphotransacetylase (*pta*) cannot produce acetic acid and cannot grow anaerobically on any of these substrates [21,22]. Mutants affecting fermentation are listed in Table 1.

As predicted by considering the redox balance [Fig. 2], eliminating both acetate and ethanol production by mutations in both *adhE* and *pta* restores the ability to ferment glucose which is defective in either mutant singly [19,22]. Such *pta adhE* double mutants rely on lactate as the major fermentation product and are restricted to growth on sugars themselves. They are unable to use either sugar alcohol or acid derivatives, since they can neither cope with more reducing equivalents than 4[H] per C6 nor with less [22].

Succinate may be formed by the condensation of phosphoenol-pyruvate with CO_2 and a subsequent reduction which consumes 4[H]/succinate [2,13]. This pathway is catalysed by phosphoenol-pyruvate carboxylase (*ppc*), malate dehydrogenase (*mdh*), *fumarase* (*fumB* and *fumC*) and fumarate reductase (*frdABCD*) [see Fig. 1 and Table 1]. Fumarase B is anaerobically induced and like fumarate reductase, under *fnr* control [23,24]. Fumarase C is formed both aerobically and anaerobically and thus probably also contributes to this pathway [23,24]. This pathway consumes −8[H] per C6 if we regard the CO_2 as 'atmospheric'. In practice the CO_2 is derived from the formate released from a second pyruvate molecule by PFL [see Fig. 2]. Each CO_2 produced is therefore matched by an acetyl-CoA which is ultimately converted to acetate or ethanol. Hence, the carbon balance requires that one acetate or ethanol be produced for each succinate formed. However succinate might also be formed by condensation of two acetyl-CoA units via the glyoxylate cycle [13,25,26]. This would result in the *production* of one NADH per succinate i.e. +2[H] per C6. The glyoxylate cycle is normally severely repressed by the presence of sugars and only functions during aerobic growth on acetate or fatty acids [25,26]. Hence in wild type strains fermenting sugars anaerobically succinate comes almost entirely from a $C_3 + C_1$ condensation. However in certain anomalous strains where succinate is a major fermentation product some of the succinate may be derived from a $C_2 + C_2$ condensation [22]. The fact that fumarate reductase mutants are not auxotrophic for succinate anaerobically implies that at least sufficient succinate for biosynthetic purposes derives from a pathway not involving fumarate. However several possibilities exist, including basal levels of isocitrate lyase or of succinate dehydrogenase acting in reverse [27].

Succinyl-CoA synthetase negative mutants will not grow anaerobically unless supplemented with methionine and lysine, both of which require succinyl-CoA as a biosynthetic precursor [28]. Hence there is no significant generation of succinyl-CoA anaerobically via the alpha-ketoglutarate dehydrogenase complex.

4. EFFECT OF CARBON SOURCE

As remarked above, an alteration of the oxidation level within a series of related glucose derivatives results in a corresponding alteration in the proportions of fermentation products of different oxidation levels. When pentoses, such as arabinose, or disaccharides, such as maltose, are used as fermentation substrates the same mixture of products is found as with glucose. However the proportion of lactate is greatly decreased and there is a corresponding increase in ethanol plus acetate such that the redox balance is preserved [18]. Little further information is available on this topic. In the case of deoxyhexoses such as fucose or rhamnose a novel fermentation pathway produces the fermentation product 1,2-propanediol [29–31].

Those members of the *Enterobacteria*, such as *Klebsiella*, which can ferment glycerol also use a unique pathway involving a cobamide-mediated rearrangement [32–34]. In theory, *E. coli* could convert glycerol to ethanol plus formate. To achieve this, and maintain redox balance, it would be necessary to switch off the phosphotransacetylase/acetate kinase and lactate pathways completely. In practice the PTA-ACK pathway is constitutive and LDH appears to respond primarily to pH. Possibly this is why *E. coli* does not grow anaerobically on glycerol alone. The detailed consideration of these alternate pathways is beyond the scope of this review.

5. ENERGY BALANCE

This review is written from the viewpoint of balancing reducing equivalents and the problem of energy generation is considered only incidentally in each section. To summarize briefly, the conversion of acetyl-CoA to acetate by acetate kinase is accompanied by the synthesis of ATP [Section 10], whereas ethanol production results in loss of the energy of the acetyl-CoA thioester. In practice the acetate : ethanol ratio is determined by the redox balance, and the cell cannot increase acetate production to increase ATP generation. The excretion of lactic acid generates an H^+ gradient [35]. The H^+ : lactate ratio is uncertain and so therefore is the yield of ATP [35,36]. The production of (C_3 + C_1) succinate is energy neutral if synthesized via the malic enzymes but wastes a high energy phosphate if the phosphoenolpyruvate to oxaloacetate route is used. The net yield of ATP during fermentation is thus 2 ATP/glucose from glycolysis and, on average, an additional 1 ATP/glucose from the operation of the lactate or acetate pathways.

6. REGULATION

Many genes involved in anaerobic respiration are regulated by the transcriptional activator gene *fnr* [37–39]. However the enzymes of fermentation are not generally affected by fnr. There are two exceptions of present interest. First, pyruvate formate lyase (*pfl* gene) is under partial *fnr* control (see Section 8). Secondly, nickel uptake is regulated by *fnr* and the activities of the various hydrogenases which require Ni^{2+}, are consequently affected [40–42]. There are several hydrogenase isoenzymes in *E. coli* all of which are anaerobically induced [42,43]. However presently available *hyd* mutants affect all three isoenzymes [44–46]. At present no general regulatory mechanism has been found which controls the fermentation pathways.

7. LACTIC ACID

There are three lactate dehydrogenase (LDH) enzymes in *E. coli* which interconvert lactic and pyruvic acids. Two membrane bound flavoproteins, one for the D- and the other for the L-isomer, convert lactate to pyruvate during oxidative growth on lactate [47]. These enzymes are not involved in fermentation and are unidirectional in vivo [47,48].

There is a single fermentative lactate dehydrogenase which is soluble and NAD-linked [49]. It is also more or less unidirectional in vivo and serves to convert pyruvate to lactate during fermentation. Originally this enzyme was mistakenly thought to produce the L-isomer [49] but later work showed that D-lactate is the isomer synthesized [11]. The fermentative LDH is present both aerobically and anaerobically. Its level increases anaerobically by 5 to 10-fold, but only upon substantial acidification of the medium. In air, such acid induction does not occur [20]. The consequence of this is that the proportion of lactate relative to acetate/ethanol/formate is higher the more acidic the medium during anaerobic fermentation [3,15–17]. In effect the cell is replacing two formic acid, one acetic acid and one ethanol with two molecules of lactic acid (see Fig. 1). Hence there is a net slowing down in further acidification of the medium.

The fermentative LDH is allosterically regulated and its activity increases hyperbolically with increased pyruvate concentration [12]. When pyruvate concentrations are low the enzyme is barely active. Only when substantial pyruvate

levels accumulate will the LDH convert a significant fraction of the pyruvate to lactic acid [12]. This fact, together with the presence of substantial LDH levels aerobically, argues that this enzyme has an overspill function. When aerobic cultures are shifted to anaerobic conditions pyruvate dehydrogenase is rapidly inactivated by the build-up of NADH due to cessation of the respiratory chain [9,14]. A rapid build-up of pyruvate ensues, and the presence of LDH would allow disposal of the excess without waiting for induction of the other fermentative pathways. During normal aerobic growth pyruvate never rises to a level sufficient to activate the LDH and is therefore not wasted.

Mutants lacking the fermentative LDH (*ldhA*) have been isolated and mapped at 30.3 minutes just clockwise of the *rac* prophage [20]. Such mutations have no growth phenotype either aerobically or anaerobically when present alone. However, a double *pfl ldhA* mutant cannot grow on glucose plus acetate [20]. Although *pfl* mutants cannot grow anaerobically on glucose alone they can grow if acetate is provided for biosynthesis [50]. Under such conditions *pfl* mutants generate energy by lactate fermentation. Introduction of an LDH defect blocks this remaining fermentation pathway. Not all of the LDH defective mutations map at the *ldhA* locus and there is a second locus, *ldhB*, so far unmapped [Mat-Jan, unpublished].

Mutants of *E. coli* deficient in ADH cannot grow anaerobically on glucose despite the presence of *E. coli* LDH. However a multicopy plasmid carrying the *Clostridium acetobutylicum* gene encoding LDH does allow *E. coli adhE* mutants to grow under these conditions (Contag and Rogers personal communication). This implies that competition between LDH and PFL for their common substrate, pyruvate, is critical. Excess LDH consumes sufficient pyruvate that any imbalance in the conversion of acetyl-CoA to ethanol/acetate becomes irrelevant.

8. FORMIC ACID AND PYRUVATE FORMATE LYASE

The anaerobic alternative to the conversion of pyruvate to lactate is its conversion to acetyl-CoA plus formate by pyruvate formate lyase (PFL) [2,10]. The regulation of PFL is rather complex. A moderate basal level of PFL is present in aerobic cells in an inactive form [10]. Upon shift to anaerobic conditions the inactive form of PFL is converted to the active enzyme by a complicated reaction requiring reduced flavodoxin and *S*-adenosyl methionine [51,52] and catalysed by the PFL activating enzyme. It is thought that activation involves creation of a free radical at the active site [53]. In addition to activation of pre-existing enzyme, the synthesis of PFL is regulated at the transcriptional level. The *pfl* gene is induced anaerobically and is under partial *fnr* control [54]. However, *fnr* mutants grow anaerobically on glucose as there is still substantial expression of *pfl* in the absence of the Fnr activator protein. The *pfl* gene is preceded by multiple promoters which are presumably responsible for this multifaceted regulation [54].

The acetyl-CoA produced by PFL may be converted to acetate or ethanol (see below) and the formic acid may be broken down further to give hydrogen and carbon dioxide, by formate hydrogen lyase (FHL) [2]. Consideration of FHL is beyond the scope of this review, except to note that FHL is induced anaerobically both by its substrate formate and also by increased acidity [16,55,56]. In this last property, it resembles the fermentative LDH (see section 7). The ultimate fate of the H_2 and CO_2 from formate is also worth considering. It appears from the examination of fermentation balances that part of the H_2 may be recycled to increase the output of ethanol relative to acetate, since the yield of ethanol is sometimes higher than expected from the simple scheme of Fig. 1. Whether reducing equivalents are recirculated internally or whether hydrogen gas is first released and then taken up again is unknown. This phenomenon varies considerably with both strain and growth substrate [18]. Carbon dioxide is also recycled to some extent, via phosphoenolpyruvate carboxylase and the two malic enzymes, which can each convert a 3 carbon intermediate to a 4 carbon molecule as discussed above.

9. ALCOHOL

The most highly reduced major fermentation product of *E. coli* is ethanol [57]. Acetyl-CoA is converted to ethanol in two steps. Acetaldehyde CoA dehydrogenase (ACDH) converts acetyl-CoA to acetaldehyde [58,59]:

$$CH_3CO\text{-}SCoA + NADH + H^+ \rightarrow CH_3CHO + NAD^+ + CoASH$$

Alcohol dehydrogenase (ADH) then converts acetaldehyde to ethanol, consuming a second reduced NADH [60,61]:

$$CH_3CHO + NADH + H^+ \rightarrow CH_3CH_2OH + NAD^+$$

It seems likely that these two activities are due to a single protein of 96 000 daltons per monomer, making it one of the largest major polypeptides encoded by *E. coli*. Gel filtration gives a molecular weight of just over 200 000 indicating that a dimer is the native structure [Koepke and Clark, in preparation]. The two enzyme activities copurify through a variety of steps [58] and cosediment during ultracentrifugation [62]. Mutations in the *adhE* gene result in loss of both activities [63,64] and it is possible to isolate temperature sensitive *adhE* mutants in which both activities are thermolabile implying that *adhE* is the structural gene for both enzyme activities [63,64].

Most alcohol dehydrogenases, both eukaryotic and bacterial, are less than half the size of the *E. coli* ADH/ACDH protein. Perhaps the most relevant comparison is between *E. coli* and *Clostridium kluyveri*, which has separate alcohol and CoA-linked acetaldehyde dehydrogenases. Both enzymes have a low molecular weight but in vivo they form a tightly associated complex, which is functionally equivalent to the large dual activity enzyme of *E. coli* [65,66]. In yeast and *Zymomonas*, pyruvate is converted directly to acetaldehyde by pyruvate decarboxylase [2] and there is no enzyme corresponding to the CoA linked acetaldehyde dehydrogenase of *Enterobacteria* and *Clostridia*, although all have ADH activities.

In addition to the *adhE* enzyme, there are two other ADH activities in *E. coli* [see 67,68]. One is due to the NAD linked propanediol dehydrogenase [31,69]. This enzyme interconverts lactaldehyde and propanediol as part of the fermentation pathway for the methylpentoses fucose and rhamnose. However it shows substantial activity toward ethanol, ethylene glycol, and other similar alcohols [69]. The *E. coli* propanediol dehydrogenase is homologous to the *Zymomonas* ADH isoenzyme II [70,71]. Both enzymes are activated by Fe^{2+} rather than the more usual Zn^{2+}, and they share limited homology with other ADH enzymes.

E. coli has an NADP linked ADH activity which oxidizes long chain alcohols [72]. This enzyme has been postulated to function in lipid biosynthesis [73]. Other NADP-linked ADH activities using butanol and allyl alcohol as substrates have also been reported [74–76]. Whether these represent activities of one non-specific enzyme or whether there are multiple NADP linked enzymes is unknown because the enzyme(s) has not been purified, nor have any mutants been isolated. No significant NADP-linked activity is detected with ethanol itself [Worland and Clark, unpublished].

The fermentative ADH is encoded by the *adhE* gene at 27 minutes on the chromosome and is synthesized only under anaerobic conditions and in the absence of nitrate [61]. Mutations giving rise to high levels of ADH synthesis, even in the presence of oxygen or nitrate, map next to *adhE* at the *adhC* locus [6]. The *adhC* mutations are *cis*-dominant and probably affect a promoter region rather than a regulatory protein. Mutations in *adhR* at 72 minutes increase the expression of ADH by around 10-fold [77]. In an *adhC adhR* double mutant levels of ADH are 500 to 1000-fold elevated such that ADH becomes one of the major proteins of the cell.

Although the fermentative ADH works well with ethanol, *n*-propanol and *n*-butanol it shows only poor activity with pentanol and is ineffective with methanol or branched alcohols. ADH converts the unsaturated allyl alcohol ($CH_2 = CHCOOH$) to the highly toxic aldehyde acrolein ($CH_2 = CHCHO$) with a K_m (towards the alcohol) little different from that for ethanol itself. As a consequence, cells expressing ADH are killed by allyl alcohol and almost all of the resistant

mutants surviving this suicide selection lack both ADH and ACDH, and have mutations in the *adhE* gene [63,64]. Mutants lacking ADH/ACDH are unable to grow anaerobically when the sole carbon source is a sugar or sugar alcohol [63]. They can still ferment glucuronic acid since acetic acid is sufficient to balance this fermentation [22]. ADH negative mutants can still grow anaerobically on glycerol plus fumarate, nitrate or other alternative electron acceptors.

The *ana* mutation of Pascal et al. [78,79] results in a greatly decreased expression of both ADH and ACDH activities when cultures are grown in the presence of glucose. However, when non-repressive sugars (eg. xylose, maltose) are used, ADH/ACDH is expressed normally under anaerobic conditions [Clark, unpublished]. It is possible that *ana*, which maps in the *adhCE* region [79], alters the control by catabolite repression of the *adhE* gene. The *adhB* mutation at 18 minutes results in a 90% decrease in anaerobic expression of ADH/ACDH and loss of the ability to grow on sugar alcohols anaerobically [Gupta and Clark, in preparation]. The *adhB* gene is under *adhR* control, like *adhE*, but its precise role is still obscure.

The *acd* mutation leads to a lack of ACDH activity but ADH is retained [80]. Originally the *acd* gene was thought to be the structural gene for ACDH but later work demonstrated that the *acd* mutation also prevents growth on respiratory substrates such as succinate or malate [63]. It is now assumed that the ACDH active site is somehow specifically inactivated in *acd* mutants, but the basis for this is puzzling.

The *adhE* gene has been cloned and sequenced [81]. The most intriguing feature is a long open reading frame in the antisense strand whose codons are in register with the ADH coding sequence. Whether an antisense mRNA or protein is involved in anaerobic regulation is still to be determined. The sequence of the *adhE* enzyme is not homologous to any other ADH for which data is presently available.

10. ACETIC ACID

Acetyl-CoA is converted to acetyl phosphate by phosphotransacetylase (PTA) [82]:

$$CH_3CO\text{-}SCoA + Pi \rightarrow CH_3CO\text{-}P + CoASH$$

Acetyl phosphate can transfer its high energy phosphate to ADP so generating ATP in a reaction catalysed by acetate kinase (ACK) [83,84]:

$$CH_3CO\text{-}P + ADP \rightarrow CH_3COOH + ATP$$

This pathway produces acetic acid and also generates ATP. Although only a single ATP per glucose is obtained, this is significant when compared with the net gain of only 2 ATP/glucose realized from the glycolytic pathway. The enzymes PTA and ACK are expressed independently of the presence of oxygen and are almost constitutive, with only a two-fold variation in level depending on the medium composition [82]. When E. coli grows on glucose in air the majority of the acetyl-CoA is converted to acetate via the PTA/ACK pathway and only a minority is metabolized via the Krebs cycle to give NADH and CO_2. Thus acetate accumulates in the medium in large amounts even when oxygen is present, although aerobic cells growing with glucose produce negligible amounts of the other fermentation products. When the glucose supply is exhausted there is a brief lag period, after which acetate is taken back into the cells and respired. Such aerobic uptake of acetate also occurs mainly via the PTA/ACK pathway [13,26].

Another enzyme which interconverts acetate and acetyl-CoA is the acetyl-CoA synthetase (ACS) [82]:

$$CH_3COOH + ATP + CoASH \rightarrow CH_3CO\text{-}SCoA + AMP + PPi$$

This enzyme is induced by acetate and operates via an acetyl-adenylate intermediate [82]. Its presence probably accounts for the ability of mutants defective in PTA and/or ACK to grow slowly in air with acetate as carbon source.

Anaerobically, glucose is converted to a mixture of fermentation products of which acetate is a major component [15–17]. The proportion of acetate depends on the nature of the sugar derivative being fermented as discussed above. Mutants lacking PTA cannot grow anaerobically on minimal medium with glucose or other sugars as

sole carbon source, nor can they grow with glycerol plus fumarate [85]. However, mutants defective in ACK, but which retain PTA, do grow anaerobically [85]. It appears that acetyl-phosphate can transfer its phosphate to Enzyme I of the phosphotransferase system, and hence to glucose [86]. Transfer of this high energy phosphate to Enzyme I of the phosphotransferase system would generate free acetate without the intervention of ACK.

Mutations affecting phosphotransacetylase (*pta*) and acetate kinase (*ackA*) map at 50 minutes [85,87]. The *pta* and *ackA* genes form an operon and evidence from Mud and Tn*10* insertions in *Salmonella* indicates that *ackA* is promoter proximal [88,89]. Whether *pta* and *ackA* are the structural genes is unclear. Temperature sensitive mutants have been isolated [90]. However, the results derived using these mutants have never been confirmed, and in particular the recently shown connection between acetyl-phosphate and the PTS system [86] largely invalidates the conclusions of this earlier work. The situation is complicated by two other observations. Firstly, mutations resulting in a lack of ACK and mapping at 39 minutes have been isolated and designated *ackB* [79]. Secondly, mutations affecting the acetyl-CoA synthetase activity (*acs*) have been mapped to the *pta ackA* region at 50 minutes [91]. It is possible that *acs* corresponds to the *facB* mutation of Guest [85]. The *facB* mutants were isolated, together with *pta, ackA* and *facA* (lacking both ACK and PTA) by selecting for resistance to fluoroacetate. All these mutations map very close together, yet *facB* mutants retained ACK and PTA activities and were still able to grow anaerobically [85]. The presence or absence of ACS was not investigated in this earlier work, and the exact relationships between *facA*, *facB*, *pta*, *ackA* and *acs* therefore remain unclear.

The build up of acidic products, particularly acetic acid, eventually inhibits further growth of anaerobic cultures, probably by acting as a weak uncoupler [92]. At pH 4.5 the level of acetate inside the cell is several times its external concentration, at least in *Clostridia* [93]. These observations have been used as the basis for a selection procedure which kills acid-excreting cells [94]. This allows the survival of mutants which produce little or no acidic fermentation products and yields a variety of mutations including those in *pta/ack* [94].

11. CONCLUSIONS

The fermentation products and pathways of enteric bacteria have been known for a long time [95,96]. However it is only relatively recently that the genetics and regulation of the pathways have been studied. The genes and corresponding enzymes involved in fermentation are summarized in Table 1 and Fig. 3. It is apparent that it is not sufficient to follow just the flow of carbon skeletons but that the reducing equivalents generated in glycolysis must be balanced by adjusting the fermentation product mix appropriately. An understanding of the regulation of these deceptively simple pathways remains a future hope.

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