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Responses of lactic acid bacteria to oxygen *

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1. SUMMARY

A small number of flavoprotein oxidase enzymes are responsible for the direct interaction of lactic acid bacteria (LAB) with oxygen; hydrogen peroxide or water are produced in these reactions. In some cultures exposed to oxygen, hydrogen peroxide accumulates to inhibitory levels.

Through these oxidase enzymes and NADH peroxidase, O_2 and H_2O_2 can accept electrons from sugar metabolism, and thus have a sparing effect on the use of metabolic intermediates, such as pyruvate or acetaldehyde, as electron acceptors. Consequently, sugar metabolism in aerated cultures of LAB can be substantially different from that in unaerated cultures. Energy and biomass yields, end-products of sugar metabolism and the range of substrates which can be metabolised are affected.

Lactic acid bacteria exhibit an inducible oxidative stress response when exposed to sublethal levels of H_2O_2. This response protects them if they are subsequently exposed to lethal concentrations of H_2O_2. The effect appears to be related to other stress responses such as heat-shock and is similar, in some but not all respects, to that previously reported for enteric bacteria.

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2. INTRODUCTION

In this paper the term Lactic Acid Bacteria (LAB) is interpreted in the traditional way i.e. the four genera *Streptococcus* (*S.*), *Leuconostoc* (*Leuc.*), *Pediococcus* (*P.*) and *Lactobacillus* (*L.*). The group includes some strict anaerobes but the majority of strains investigated are aerotolerant to some degree, and often completely so. With very few exceptions, LAB react to O_2 and the consequences of such reactions may be beneficial or detrimental. LAB generally grow satisfactorily in the absence of O_2, and in its presence some are inhibited partially or completely. Consequently, it is quite usual to consider that the normal growth metabolism of LAB is anaerobic and that metabolism in the presence of O_2 is somewhat aberrant.

3. ENZYMATIC REACTIONS OF LACTIC ACID BACTERIA WITH OXYGEN

In general, LAB remove O_2 from solution, often at substantial rates when their environment contains a substrate which they can oxidise. The oxidation–reduction reactions involve a transfer of 1, 2 or 4 electrons to the dioxygen molecule as follows:

$$O_2 + 1e^- \rightarrow O_2^-$$

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$$

The superoxide anion (O_2^-) is short-lived as it is dismuted, spontaneously or through superoxide dismutase (SOD) or high intracellular Mn^{2+}, to the more stable H_2O_2. No enzyme has been identified in LAB which produces O_2^- as an end-product but it is a possible intermediate compound in the formation of H_2O_2 or H_2O. The enzymes of LAB catalysing reactions with O_2 which have been identified, all produce H_2O_2 or H_2O as end products (Table 1). They are principally pyridine nucleotide oxidases but also pyruvate oxidase and α-glycerophosphate oxidase.

3.1. Reduced pyridine nucleotide oxidases

Possession on an NADH oxidase appears to be a universal property of LAB. Three types have been reported. The first two are non-haem flavoproteins, one of which catalyses the reduction of O_2 to H_2O_2, the other the reduction of O_2 to H_2O. In many reports of NADH oxidases in LAB, the assay system used was the oxidation of NADH and the product was not measured. The difficulty in distinguishing between H_2O and H_2O_2 forming enzymes is further exacerbated by the presence of an NADH peroxidase in many extracts, as the activity of a H_2O_2 forming NADH oxidase plus that of excess NADH peroxidase is similar to the activity of H_2O forming NADH oxidase.

The NADH: H_2O_2 oxidase (Table 1) was first reported in *S. faecalis* by Dolin [1,2] and its presence in many LAB has since been confirmed [3–8]. The enzyme is cytoplasmic, is rather unstable and has not been purified.

The NADH: H_2O oxidase (Table 1) is cytoplasmic and is also widespread in LAB [7–17]. The enzyme has been purified from *L. plantarum* [9], *S. faecalis* [10,17] and *Leuc. mesenteroides* [15]. The enzyme from *S. faecalis* has a molecular mass of 51 kDa and probably consists of a single polypeptide chain with one FAD prosthetic group [17]. The *Leuc. mesenteroides* enzyme has a molecular mass of 104 kDa and probably exists as a dimer with one FAD group per subunit [15]. The enzyme from *L. plantarum* was estimated to have a molecular mass of approximately 170 kDa with two molecules of FAD per enzyme molecule [9]. Neither O_2^- nor H_2O_2 were detected as intermediates in the oxidation of NADH by the *S. faecalis* oxidase, although Fukui [9] detected H_2O_2 as an end-product of NADH oxidation by an enzyme preparation disrupted with 2 M urea.

The third NADH oxidase is quite rare in LAB; it was observed in some LAB grown aerobically in media containing haematin. The enzyme is associated with the membrane fraction and is thought to form part of a rudimentary electron transport chain which reduced O_2 to water and promotes a weak proton extrusion [18]. This system is widespread among strains of *S. faecalis* [19], but has only been observed in a few strains of other LAB [20].

The NADH oxidases described above have a pronounced preference for NADH rather than NADPH. A non-haem protein with NADPH oxidase activity has been detected in *Peptostreptococcus anaerobius* a strict anaerobe; this enzyme catalyses the formation of O_2^- and H_2O_2 from O_2

Table 1

Reactions involving molecular oxygen or oxygen metabolites catalysed by enzymes of lactic acid bacteria

1. $NADH + H^+ + O_2 \xrightarrow{\text{NADH: } H_2O_2 \text{ oxidase}} NAD^+ + H_2O_2$
2. $2\ NADH + 2H^+ + O_2 \xrightarrow{\text{NADH: } H_2O \text{ oxidase}} 2\ NAD^+ + 2\ H_2O$
3. $\text{pyruvate} + \text{phosphate} + O_2 \xrightarrow[TPP,\ FAD]{\text{pyruvate oxidase}} \text{acetylphosphate} + CO_2 + H_2O_2$
4. $\alpha\text{-glycerophosphate} + O_2 \xrightarrow{\text{oxidase } \alpha\text{-glycerophosphate}} \text{dihydroxyacetone phosphate} + H_2O_2$
5. $2\ O_2^- + 2\ H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$
6. $NADH + H^+ + H_2O_2 \xrightarrow{\text{NADH peroxidase}} 2H_2O$

TPP = thiamine pyrophosphate; FAD = flavin adenine dinucleotide.

[21]. It is presumed that O_2^- is an intermediate in H_2O_2 formation.

3.2. *Pyruvate oxidase*

Pyruvate oxidase which catalyses a direct reaction of pyruvate with molecular O_2 (Table 1) was first demonstrated in *L. delbrueckii* [22] and since then in a few other lactobacilli [3,5,14,23] in *S. mutans* [24] and in *P. halophilus* [25]. The enzyme is cytoplasmic and was purified by Hager et al. [22] who showed that it contained FAD. Pyruvate oxidase in cell-free extracts of *L. plantarum* did not produce O_2^- as an intermediate [26].

3.3. *α-Glycerophosphate oxidase*

The reaction catalysed by α-glycerophosphate oxidase (Table 1) occurs in those LAB which utilise glycerol as an O_2-dependent growth substrate. They include enterococci [27–29], lactobacilli [3; M.G. Murphy and S. Condon, unpublished data] and *P. pentosaceus* [30]. The enzyme has been partially purified; it is cytoplasmic and contains FAD [28,29].

4. ACCUMULATION OF H_2O_2 AND OTHER POSSIBLE TOXIC O_2 METABOLITES

Hydrogen peroxide is a product of some flavoprotein oxidases of LAB with O_2 and it may accumulate in aerobic cultures of many strains [reviewed in 31]. Work at this laboratory established that about half of 20 Group N streptococci [8] and several lactobacilli [32] were inhibited by H_2O_2 accumulation during aerobic growth. Other reports clearly demonstrate that accumulation to auto-inhibitory levels during exposure to O_2 is widespread, but not universal among LAB [4,7,27,33,34; J.B. Smart and T.D. Thomas, unpublished data). Accumulation of H_2O_2 is due to greater overall activity of systems that produce H_2O_2 than those which eliminate it. The H_2O_2 forming oxidases are undoubtedly the principal mechanisms for the production of H_2O_2 in the LAB. It has been suggested that dismutation of O_2^- may also be a physiologically important source of H_2O_2 [35]. Hyperbaric O_2 or agents which increase O_2 uptake such as plumbagin undoubtedly promote extensive O_2^- production which can be dismuted to H_2O_2 by SOD (Table 1), or high internal manganese concentrations [36,37]. However, there is little convincing evidence as yet that H_2O_2 accumulation in LAB, growing under ordinary aerobic conditions, occurs via O_2^- in any substantial way. These bacteria lack many of the cellular components which promote the generation of O_2^- radicals from O_2 [38]. Only 17% of the O_2 utilised during NADH oxidation by cell-free extracts of *S. faecalis* (in which SOD was inhibited by a specific antibody) could be attributed to O_2^- formation [35]. Another report indicated that only 6% of the O_2 metabolised by whole cells of *S. mutans* (with low SOD activity) was converted to O_2^- [7]. It was expected that NADH oxidases might be a likely source of O_2^- formation [35], but O_2^- production was not detected during NADH oxidation by a purified NADH : H_2O oxidase from *S. faecalis* [17]. In addition O_2^- production was not observed during O_2 reduction by cell-free extracts of *L. plantarum* with NADH, NADPH or pyruvate [26]. Some other oxidase of LAB such as NADH : H_2O_2 oxidase may release O_2^- as an intermediate, but at present the only convincing evidence for such an enzyme is that from *Peptostreptococcus anaerobius* [21]. The widespread distribution of SOD (or non-enzymatic manganese dismutation system) among LAB [35–37,39] does not fit comfortably with these observations. It has been suggested that SOD may have other physiological functions besides dismutation of O_2^- [40], but it is also possible that even low levels of O_2^- are so reactive that an active dismutation system is an important safeguard in aerobic environments.

In this context also is the question of whether the inhibition of LAB which coincides with H_2O_2 accumulation is caused directly by H_2O_2 or indirectly by a metabolite of H_2O_2. Inhibition of LAB by aeration at normal atmospheric pressures is invariably prevented by catalase [reviewed in 31;6,7,41,42]. However, it has been suggested that H_2O_2 can react with O_2^- to form hydroxyl radical ($OH\cdot$) and that the latter is the direct inhibitor of O_2-sensitive cells [43]. Although the feasibility is

not universally accepted [40] formation of OH· from H_2O_2 and O_2^- under physiological conditions has been demonstrated [44]. As already mentioned, the rate of production of O_2^- by LAB is probably low under normal aeration conditions. If OH· is the direct toxic O_2 metabolite, it would have to be effective at concentrations much lower than those of H_2O_2, which accumulates in aeration-inhibited cultures of LAB. Some years ago work at our laboratory showed that H_2O_2 added to sterilised milk, at the concentrations which inhibited Group N streptococci, clearly inhibited acid production by unaerated milk cultures of the same bacteria [45]. Addition of mannitol, a known scavenger of OH· [43], failed to relieve aeration inhibition of an *S. lactis* strain whereas catalase did [46]. At least for Group N streptococci, therefore, direct inhibition by H_2O_2 rather than OH· remains the most likely explanation for O_2 sensitivity.

The inhibitory effects of H_2O_2 on LAB may be potentiated in natural environments, such as milk or saliva; concentrations which are not of themselves inhibitory, may become so, because of the presence of lactoperoxidase and thiocyanate. Hydrogen peroxide produced by oral [47], mastitic [41], or Group N [48] streptococci may oxidise thiocyanate to hypothiocyanite in a reaction catalysed by lactoperoxidase as follows:

$$SCN^- + H_2O_2 \xrightarrow{\text{lactoperoxidase}} OSCN^- + H_2O$$

Further oxidation products formed in the presence of excess hydrogen peroxide (O_2SCN^- and O_3SCN^-) may also be involved in the inhibition caused by the lactoperoxidase-thiocyanate-H_2O_2 system but most attention has been focussed on $OSCN^-$ [reviewed in 49]. The mode of action of hypothiocyanite is complex and not fully understood. The overall effect on LAB is generally bacteriostatic whereas many Gram-negative bacteria are rapidly killed in its presence. A number of different physiological effects have been noted, principally leakage of K^+ and amino acids, inhibition of uptake of carbohydrates, lactate and amino acids and the inhibition of specific glycolysis enzymes such as aldolase, hexokinase and glyceraldehyde-3-phosphate dehydrogenase. Streptococci resistant to hypothiocyanite have an NADH : $OSCN^-$-oxidoreductase which reduces $OSCN^-$ to the inert SCN^- [49].

Accumulation of H_2O_2 in aerobic cultures in LAB results from a greater capacity to form H_2O_2 than to break it down. A flavoprotein NADH peroxidase (Table 1) first reported by Dolin [2,50] and which is widespread among LAB [3–5,12,14, 47,51,52; J.B. Smart and T.D. Thomas, unpublished data] is probably mainly responsible for H_2O_2 breakdown.

The enzyme purified from *S. faecalis* contained FAD [2]. It was not possible to separate the peroxidase from NADH oxidase in some LAB [5,12, J.B. Smart and T.D. Thomas, unpublished data]. In *L. plantarum* cultures NADH peroxidase was induced by O_2 [14] and catabolite repressed by glucose [53,54]. Other enzymes which reduce H_2O_2 have been found in a few strains of LAB. Both non-haem catalases termed pseudo-catalases and haem-catalases have been reported [reviewed in 31]. The latter enzymes require aerobic growth in the presence of haematin as only the apoenzyme is made by the LAB [55].

5. AERATION-INDUCED CHANGES IN SUGAR METABOLISM

When growing in the absence of O_2, LAB rely mainly on lactic dehydrogenase (LDH), acetaldehyde dehydrogenase and alcohol dehydrogenase, to regenerate NAD^+ needed for the dehydrogenation reactions of sugar metabolism. In the presence of O_2, however, NADH oxidases and NADH peroxidases are alternative mechanisms of NAD^+ regeneration. These mechanisms can have a sparing effect on pyruvate, acetyl-CoA and acetaldehyde and can alter the fermentation end-product spectrum.

5.1. Response of the heterolactic leuconostocs to aeration

The end-products of anaerobic glucose fermentation by leuconostocs are mainly lactate, ethanol and CO_2, but acetate is largely substituted for ethanol during aerobic growth (Fig. 1; [16, 54–58]). Strains which are missing NADH oxidase (occur-

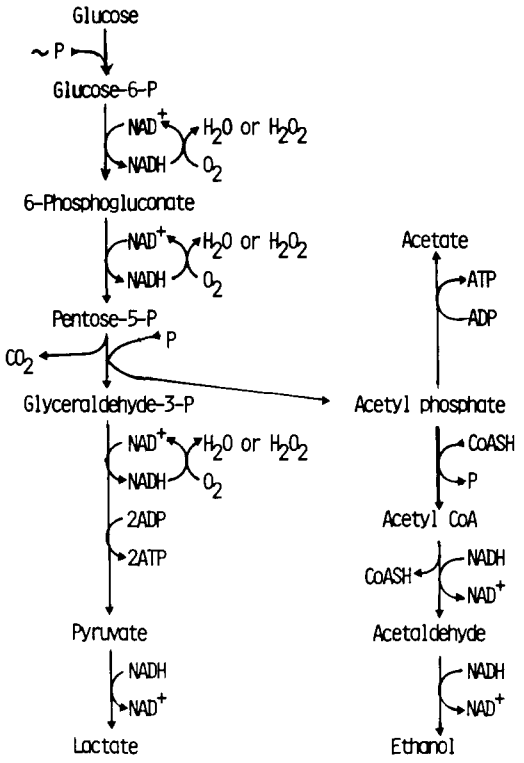

Fig. 1. Heterolactate pathway of glucose dissimilation.

ring naturally or constructed by mutation) form lactate, ethanol and CO_2 in the presence or absence of O_2 [16]. The ethanol branch of the heterolactic (phosphoketolase) pathway is needed to regenerate NAD^+ for dehydrogenation of glucose-6-phosphate and 6-phosphogluconate. The regulation of synthesis of the enzymes concerned with the alternative routes of acetylphosphate metabolism is ideally organised to take advantage of the presence or absence of O_2. When O_2 is available the specific activities of NADH oxidase and acetate kinase are high and those of phosphate acetyl transferase and alcohol dehydrogenase are low, thus facilitating acetate synthesis and high energy phosphate conservation. In the absence of O_2 the leuconostocs respond with greater amounts of phosphate acetyl transferase and alcohol dehydrogenase and less NADH oxidase and acetate kinase activities [16].

The switch from ethanol to acetate synthesis doubles the amount of ATP that is formed from glucose (Fig. 1). The molar growth yield of *Leuc. mesenteroides* (NADH oxidase-positive) growing aerobically on glucose is approximately double that obtained during anaerobic growth [16]. In addition, growth rates on glucose in the presence of O_2 are much faster than in its absence [16,56], indicating that the rate of ATP formation from sugars is a growth-rate-limiting step in these bacteria. The RNA/DNA and RNA/protein ratios of *Leuc. mesenteroides* cells grown aerobically or anaerobically on glucose are similar, which indicates that the macromolecular composition of these bacteria does not change, even though the growth rate is faster in the presence of O_2 [M. O'Keeffe and S. Condon, unpublished data].

Oxygen is not the only electron acceptor which stimulates leuconostocs growing on glucose. Growth of several strains are also stimulated by other exogenous substrates of dehydrogenase enzymes, such as pyruvate or acetaldehyde. Citrate also stimulates some strains, presumably, because it serves as a source of additional pyruvate [59].

5.2. O_2-induced alterations in pyruvate transformation in lactic acid bacteria classified as homofermentative

The EMP pathway of sugar metabolism is used by streptococci, pediococci and some lactobacilli. In unaerated (not necessarily strictly anaerobic) cultures, with excess glucose as substrate, most of the pyruvate is converted to lactate to reoxidise NADH. Many of these bacteria have the genetic capacity to make products other than lactate from pyruvate, such as acetate, CO_2, formate, ethanol, acetoin, diacetyl and 2,3-butanediol [see Figs. 2–4] and their accumulation can be demonstrated by varying the conditions of sugar utilisation. For example, several lactic streptococci accumulate substantial amounts of formate, ethanol and acetate and correspondingly less lactate when grown in glucose- or lactose-limited unaerated chemostat cultures, whereas with excess sugar the same cultures are homolactic. The production of products other than lactate in the sugar-limited cultures is attributed to relatively low intracellular levels of fructose-1,6-bisphosphate (FBP), an essential activator of lactate dehydrogenase in

these strains [60]. The presence of O_2 often has significant effects on the metabolism of pyruvate in these and other LAB.

5.2.1. Pyruvate to lactate transformation

Accumulation of products other than lactate from sugars by homofermentative LAB requires an alternative mechanism to lactate dehydrogenase (LDH) to oxidise NADH, or an alternative source of pyruvate which does not involve reduction of NAD^+. Most LAB have the capacity to oxidise NADH with O_2 through the NADH oxidase/NADH peroxidase system(s), which are often induced by O_2 [6,11,14,47,61; J.B. Smart and T.D. Thomas, unpublished data], or H_2O_2 [14; M. Tangney and S. Condon, unpublished data]. These enzymes may also be repressed during growth in the presence of excess glucose [6,14,53]. The LDH has to compete with the NADH oxidase/NADH peroxidase enzymes for NADH in aerobically growing cells and in some strains the concentration of LDH may be reduced, though rarely by substantial amounts (J.B. Smart and T.D. Thomas, unpublished data). A more significant effect in determining the fate of pyruvate in some LAB under aerobic conditions, may be the intracellular levels of FBP. In experiments with *Staphylococcus epidermidis*, low intracellular levels of FBP correlated closely with acetate production in aerobically growing cells, or aerobic incubation of resting cells in glucose; in anaerobic conditions high FBP levels correlated with lactate formation [62]. However, other factors besides FBP levels are involved in determining whether pyruvate is converted to lactate or to other metabolites (see below).

5.2.2. The pyruvate formate-lyase pathway to formate, acetate and ethanol

This pathway (Fig. 2) appears to be a major alternative pathway to the LDH pathway in many homofermentative LAB growing under anaerobic conditions. The end-products are formate, ethanol and acetate. The operation of this pathway varies among LAB. Thomas et al. [60] found that most Group N streptococci produced formate, acetate and ethanol from glucose or lactose in unaerated chemostat cultures only when the sugar was limiting and FBP levels were low. However, some oral streptococci accumulate these end-products even when glucose is in excess in strictly anaerobic conditions. Washed cell suspensions of anaerobic glucose cultures of *S. mutans* accumulated substantial amounts of formate, acetate and ethanol, as well as lactate, under strictly anaerobic conditions from excess glucose or galactose. The fermentation became much more homolactic in the presence of O_2, but the FBP levels were similar in the presence or absence of O_2 [63,64]. In chemostat cultures with limiting glucose, *S. mutans* and *S. sanguis* accumulated very little lactate. In the absence of O_2, formate, acetate and ethanol were produced in a 2 : 1 : 1 ratio. A gradual increase in the aeration level led to a gradual increase in acetate and a decrease in formate and ethanol [65]. The main reason for these effects is the extreme sensitivity of pyruvate formate-lyase to O_2 [63,64,66–68]. Cells of *S. mutans* exposed to O_2 for 2 min lost 94% of their activity [63]. The levels of pyruvate formate-lyase and its inhibitor glyceraldehyde-3-phosphate are thought to play key roles in regulating pyruvate metabolism in oral streptococci under anaerobic conditions [63,64,69].

5.2.3. The pyruvate dehydrogenase pathway to acetate, ethanol and CO_2

This pathway (Fig. 2) differs from the pyruvate formate-lyase pathway only in the first step, the pyruvate dehydrogenase step, in which pyruvate is decarboxylated to form acetyl CoA with the reduction of NAD^+. This enzyme has been demonstrated in several Group N streptococci [70,71; J.B. Smart and T.D. Thomas, unpublished data]. It probably co-exists with pyruvate formate-lyase in many LAB. Pyruvate dehydrogenase is not sensitive to O_2 and it is present at greater concentrations in aerobic than in anaerobic cultures (J.B. Smart and Thomas, T.D. unpublished data). In the absence of O_2, LDH and pyruvate formate-lyase have a distinct advantage over pyruvate dehydrogenase in competition for pyruvate as the latter enzyme involves reduction of NAD^+. However, in the presence of O_2, NADH can be reoxidised with the NADH oxidase/peroxidase system(s), which makes catalysis of pyruvate to

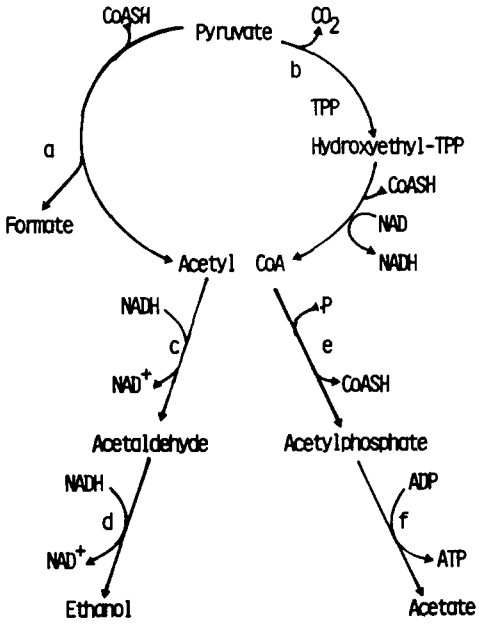

Fig. 2. Pyruvate formate-lyase and pyruvate dehydrogenase pathways a, pyruvate formate-lyase; b, pyruvate dehydrogenase; c, acetaldehyde dehydrogenase; d, alcohol dehydrogenase; e, phosphateacetyl transferase, f, acetate kinase.

acetyl CoA and CO_2 more favourable, especially since pyruvate formate-lyase is O_2-sensitive.

The acetyl CoA formed in aerobically metabolising cells is more likely to be converted to acetate via acetyl phosphate than to ethanol via acetaldehyde. The ethanol branch dehydrogenase enzymes require NADH and are in competition with NADH oxidase/peroxidase, whereas acetyl CoA converted to acetate results in ATP synthesis. Some Group N streptococci which have the pyruvate formate-lyase [67] and the pyruvate dehydrogenase [70,71] pathways, and other homolactic LAB which have not yet been checked for these activities, accumulate lactate, formate, acetate and ethanol when metabolising sugars anaerobically, and lactate, acetate and CO_2 when O_2 is available [41,72–76; J.B. Smart and T.D. Thomas, unpublished data].

5.2.4. The pyruvate oxidase pathway to acetate and CO_2

This pathway (Fig. 3) observed in a few species of lactobacilli [3,5,14,22,23], *S. mutans* [24] and *P. halophilus* [25] is an alternative mechanism to pyruvate dehydrogenase/phosphate acetyl transferase for the formation of acetyl phosphate in aerobically growing cells. Pyruvate oxidase has not been observed in Group N streptococci [8; J.B. Smart and T.D. Thomas, unpublished data]. In *L. plantarum*, this activity is induced by aeration and catabolite repressed by glucose [14,53].

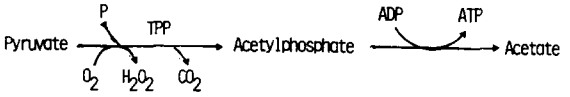

Fig. 3. Pyruvate oxidase pathway.

5.2.5. Transformation of pyruvate to acetoin, 2,3-butanediol and diacetyl

These C4 end-products of sugar metabolism (Fig. 4) are found in cultures of some LAB and their synthesis is affected by O_2 availability. The ability to make some C4 compounds, demonstrated by incubating cell suspensions, or cell-free extracts, with pyruvate is fairly widespread among LAB [20,70,71,77–79]. However, accumulation to any substantial extent in growing cultures of LAB requires special circumstances.

In general, when C4 compounds accumulate in growing cultures pyruvate, or an alternative source of pyruvate such as citrate, is available in addition to the fermentable sugar [54,78,80–83]. Neverthe-

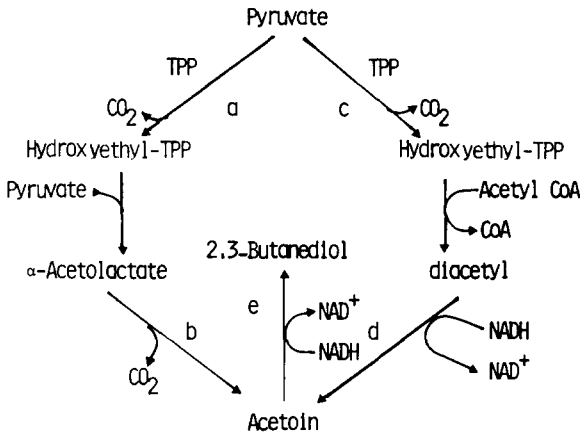

Fig. 4. Acetoin, diacetyl and 2,3-butanediol pathways. a, α-acetolactate synthase; b, α-acetolactate decarboxylase; c, diacetyl synthase; d, diacetyl reductase; e, 2,3-butanediol dehydrogenase.

less, C4 compounds have been observed occasionally in cultures of some streptococci growing in a sugar-based medium without additional pyruvate [41,73,79,84,85]. Since two molecules of pyruvate are needed in such cultures to make one C4 compound, oxidation of two NADH molecules is a prerequisite for synthesis.

Accumulation is, therefore, more likely in the presence rather than the absence of O_2. A survey of Group N streptococci [79] revealed that the related strains *S. lactis* C2, AC2 and 712 produced significant quantities of acetoin in aerobic culture, but not in the absence of O_2. Accumulation was greatest in a chemically defined medium, with galactose rather than glucose or lactose as the sugar and with catalase to eliminate H_2O_2. The level of α-acetolactate synthase was 2–4-fold higher in aerobically than in anaerobically grown cells. H_2O_2 added intermittently at sublethal levels to anaerobic cultures stimulated acetoin production. Diacetyl or 2,3-butanediol did not accumulate in growing cultures, but the latter was produced from pyruvate by resting cell suspensions, grown without but not with aeration.

6. ADVANTAGES OF AEROBIC METABOLISM

The metabolism of sugars to acetate rather than lactate doubles the amount of ATP formed through substrate level phosphorylation. Those LAB having an O_2-insensitive mechanism of acetate synthesis, should be able to benefit from the availability of the extra ATP during growth in the presence of O_2, by greater biomass yields and possibly faster growth rates. Such increases have been mentioned above when dealing with the heterolactic leuconostocs. It is not surprising that some homolactic bacteria also benefit from the presence of O_2; yields of biomass during aeration are often greater than in the absence of O_2. The effects are particularly noticeable at low concentrations of sugar [41,52,86–88]. Growth rates of some homolactic LAB are faster in the presence of O_2 than in its absence, especially if catalase is present to eliminate any H_2O_2 formed [8,32].

Aerobic growth also allows some LAB to metabolise substrates such as glycerol, mannitol, sorbitol and lactate that they do not utilise in the absence of O_2 [reviewed in 31]. In our laboratory, the O_2-dependent utilisation of lactate by *L. plantarum* has been of particular interest [53,89]. The probable pathway is via dehydrogenation of lactate to pyruvate followed by oxidation to acetyl phosphate with pyruvate oxidase and finally conversion of acetyl phosphate to acetate with synthesis of ATP. The dehydrogenation step may involve both NAD-dependent and NAD-independent LDHs; the latter but not the former was induced by aeration as was also pyruvate oxidase, NADH peroxidase and NADH oxidase. The oxidases, peroxidase and acetate kinase were all repressed by glucose [53].

When *L. plantarum* is growing aerobically in a glucose broth, D- and L-lactates are the only major end-products during active growth, i.e. when the cells have low levels of lactate oxidising enzymes. When the glucose level decreases the lactate oxidation pathway is derepressed and now acetate rather than lactate accumulates. Finally, on prolonged incubation when the glucose is exhausted, the lactate is converted to acetate and thus allows *L.* plantarum to continue growing, albeit slowly, when O_2 is available, but not in its absence [14,53,54].

7. OXIDATION STRESS RESPONSE

Some recent work indicates that LAB are similar to other organisms that have been investigated, in that they respond to sublethal concentrations of H_2O_2 by inducing a protective system which helps them survive normally lethal levels of H_2O_2. In enteric bacteria the protective system consists of a set of 'stress proteins' which somehow protect the cells against H_2O_2 damage but also against damage in other stressful environments e.g. high temperatures (heat shock response) and a variety of chemical oxidants [90,91]. Two Group N streptococci exposed to a sublethal (0.5 mm) level of H_2O_2 survived a challenge with a lethal concentration (5 mm) at substantially greater rates than cultures which were not first exposed to the sublethal concentration. The H_2O_2-activated cells were also much more resistant to lethal temperatures (e.g.

55 °C). Unlike the enteric bacteria, the protective system was also induced when anaerobically growing cells were exposed to O_2. One of the cultures tested, *S. lactis* US3, does not accumulate H_2O_2 when growing aerobically. Consequently, induction of the protective system by O_2 is unlikely to be caused by prior formation of H_2O_2. NADH peroxidase and (to a lesser extent) NADH oxidase were induced during the period of O_2 activation and also when the streptococci were exposed to a sublethal temperature (heat shock), suggesting that these enzymes may have roles in stressful situations (M. Tangney and S. Condon, unpublished data).

8. CONCLUSIONS

The generalisation that growth of lactic acid bacteria in the presence of O_2 is aberrant cannot be justified. Aerobic environments may be toxic for some strains, but for many O_2 (and or H_2O_2 formed from O_2) is an important electron acceptor which permits reoxidation of reduced pyrimidine nucleotides, over and above that which occurs in anaerobic sugar metabolism. This additional capacity for oxidation of NADH allows a wider range of substrates to be used for ATP generation and also the utilisation of pathways which are dormant in anaerobic cultures. Some of these pathways permit additional ATP generation and consequently improved molar growth yields and rates with conventional sugars such as glucose.

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