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Carbohydrate metabolism in lactic acid bacteria

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The term "lactic acid bacteria" is discussed. An overview of the following topics is given: main pathways of homo- and heterofermentation of hexoses, i.e. glycolysis, bifidus pathway, 6-phosphogluconate pathway; uptake and dissimilation of lactose (tagatose pathway); fermentation of pentoses and pentitols; alternative fates of pyruvate, i.e. splitting to formate and acetate, CO_2 and acetate or formation of acetoin and diacetyl; lactate oxidation; biochemical basis for the formation of different stereoisomers of lactate.

INTRODUCTION

Conversion of carbohydrates to lactate by the lactic acid bacteria may well be considered as the most important fermentation process employed in food technology. In the earliest days of mankind spontaneous lactate fermentation already improved the keeping qualities and the hygienic status of collected food. The process was gradually improved and "domesticated" as civilization proceeded. The microbiological and biochemical foundations of lactate fermentation only became known, however, in the course of the last century. As a result, it became possible to control this process scientifically and to apply it to modern food technology.

What are lactic acid bacteria?

The term "lactic acid bacteria" gradually emerged in the years around the turn of this century (cf. Ingram, 1975). A fairly precise definition was first given by Orla-Jensen in his seminal publication of 1919. Since then, while the boundaries of this group have remained rather vague, its centre has been unequivocally

Table 1. The genera of lactic acid bacteria, their fermentation type and products

Genus (Subgenus)	Fermentation type	Main product (molar ratio)	Configuration of lactate
Streptococcus	homofermentative	lactate	L(+)
Pediococcus	homofermentative	lactate	DL, L(+)
Lactobacillus	homofermentative	lactate	
Thermobacterium	homofermentative	lactate	D(−), L(+), DL
Streptobacterium	homofermentative	lactate	D(−), L(+), DL
	heterofermentative[1]	lactate:acetate (1:1)	D(−), L(+), DL
Betabacterium	heterofermentative	lactate:acetate:CO_2 (1:1:1)	DL
Leuconostoc	heterofermentative	lactate:acetate:CO_2 (1:1:1)	D(−)
Bifidobacterium	heterofermentative	lactate:acetate (2:3)	L(+)

[1] In the case of pentose fermentation.

accepted: gram-positive, non-sporing microaerophilic bacteria whose main fermentation product from carbohydrates is lactate. Today lactic acid bacteria sensu lato comprise cocci (*Streptococcus, Pediococcus, Leuconostoc*) and rods (*Lactobacillus* (*Lb.*), *Bifidobacterium*) exhibiting different pathways of carbohydrate fermentation (Table 1, Fig. 1) with end-products that are either exclusively (homofermentative) or at least 50% (heterofermentative) lactate.

With the exception of bifidobacteria, all genera of the lactic acid bacteria are phylogenetically related as evidenced by serological (Hontebeyrie and Gasser, 1975; London et al., 1975; London, 1976) and 16S rRNA homology studies (Stackebrandt et al., 1983). Together with the bacilli they form a "supercluster" within the "*Clostridium* sub-branch" of gram-positive eubacteria, whereas the bifidobacteria, together with the Actinomycetales, belong to the second subbranch of gram-positive eubacteria (Stackebrandt et al., 1983). The separate grouping of the bifidobacteria parallels their different technological relevance. While the lactic acid bacteria sensu stricto are members of the indigenous flora of the raw material of food and are of direct use in food technology, bifidobacteria colonize the intestine of man and animals and are found in substrates contaminated with faeces (Scardovi, 1982). They are important in dairying only in as much as they are added to special milk products for therapeutical purposes (Bifidus milk; Speck, 1976).

Pathways of hexose fermentation

The three major pathways of hexose fermentation occurring within lactic acid bacteria, schematically depicted in Fig. 1, are well known and have been described in text books (cf. Gottschalk, 1979). They have in common that only hexose phosphates with gluco-configuration are attacked. However, they differ from each other in the mode of splitting the carbon skeleton thus leading to

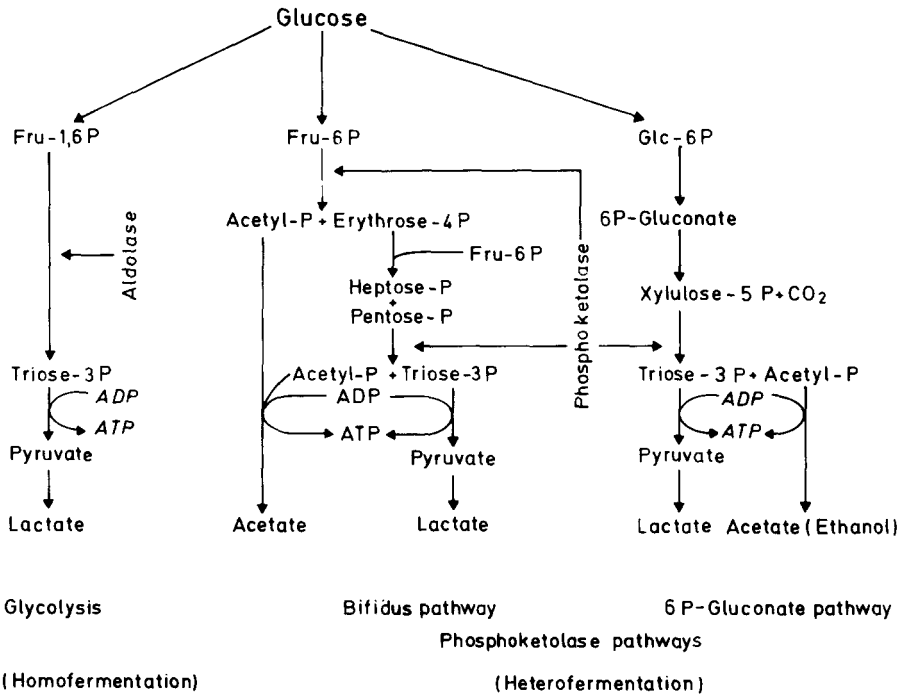

Fig. 1. Schematic presentation of the main pathways of hexose fermentation in lactic acid bacteria.

different sets of end-products. Glycolysis, occurring in streptococci, pediococci and homofermentative lactobacilli, is characterized by the splitting of fructose 1,6-bisphosphate with aldolase into two triose phosphate moieties which are further converted to lactate. Thus glycolysis leads to homolactic fermentation. Heterofermentation in leuconostocs and the so-called betabacteria is initiated by the oxidation of glucose 6-phosphate to gluconate 6-phosphate followed by decarboxylation and splitting of the resulting pentose 5-phosphate into a C-2 and a C-3 moiety. Thus equimolar amounts of CO_2, lactate and acetate or ethanol are formed from hexose. The ratio acetate/ethanol depends on the oxidation-reduction potential of the system.

If an additional hydrogen acceptor, e.g. O_2 or fructose is available, no ethanol is formed, but O_2 is reduced to H_2O_2 or H_2O and fructose is reduced to mannitol.

Heterofermentation in bifidobacteria is initiated by splitting fructose 6-phosphate with phosphoketolase into a C-2 and a C-4 moiety. While the C-2 moiety is converted to acetate, heptose 7-phosphate is formed from the C-4 moiety and a triose moiety derived from an additional molecule of fructose 6-phosphate by the action of transketolase. Consecutive splitting of the heptose 7-phosphate and the resulting pentose 5-phosphate by phosphoketolase finally results in the formation of acetate and lactate at a molar ratio of 3:2.

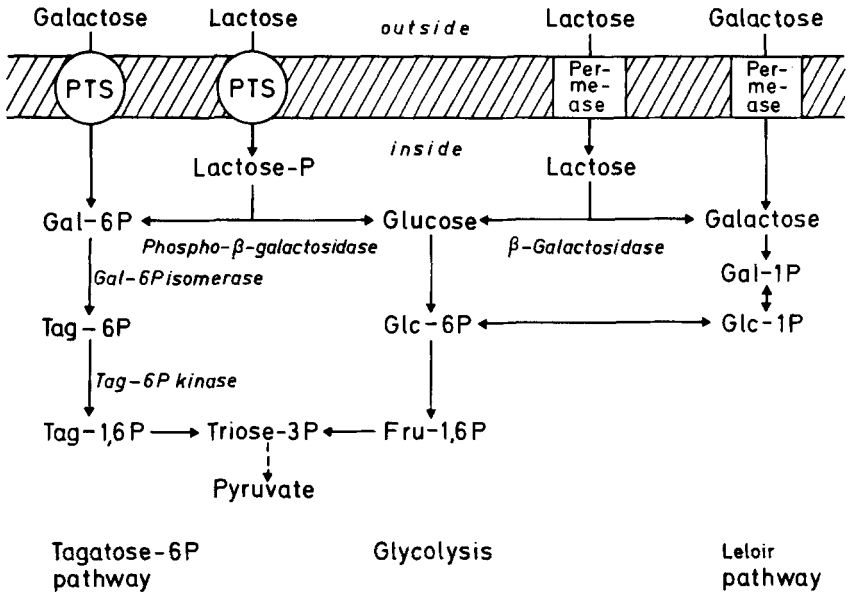

Fig. 2. Scheme of lactose and galactose uptake and dissimilation in some lactic acid bacteria.

According to these mechanisms homolactic fermentation and the two different types of heterofermentation may very easily be distinguished by the determination of the fermentation end-products and tests for typical enzymes. The results are, however, confusing, if complex substrates are fermented containing compounds other than fermentable hexoses, e.g. pentoses or organic acids yielding lactate, acetate and CO_2 in different ratios. This is often the case with natural material, i.e. fruit juices, vegetables, etc. Pyruvate may, moreover, not only be reduced to lactate but also converted to several other products by alternative mechanisms depending on the growth conditions and properties of the particular organism. These complications will be discussed below.

Lactose uptake and fermentation

The majority of saccharides and oligosaccharides are taken up by most lactic acid bacteria with the help of specific permeases and are phosphorylated inside the cell. Oligosaccharides are split by the respective glycosidases, prior to phosphorylation of the resulting monosaccharides. Accordingly, lactose is taken up by a specific permease and is split by β-galactosidase in most lactobacilli (Premi et al., 1972; cf. Lawrence and Thomas, 1979). As depicted in Fig. 2, the resulting D-galactose is converted to glucose 6-phosphate by the well-known Leloir pathway and, together with the glucose moiety of lactose, is fermented via glycolysis.

Also free D-galactose, taken up from the medium by permease, follows the same pathway. However, in most streptococci and a few lactobacilli (e.g. *Lb. casei*) lactose as well as galactose are taken up by the action of the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) (cf. McKay et al., 1970; Postma and Roseman, 1976; Thompson, 1979). The lactose phosphate formed is hydrolysed by phospho-β-galactosidase (Johnson and McDonald, 1974), resulting in glucose and D-galactose 6-phosphate (Thompson, 1980). While glucose is further fermented via glycolysis, D-galactose 6-phosphate is converted to D-tagatose 6-phosphate by D-galactose 6-phosphate isomerase, then further phosphorylated by D-tagatose 6-phosphate kinase to D-tagatose 1,6-bisphosphate and split to triose phosphate by D-tagatose 1,6-bisphosphate aldolase (D-tagatose 6-phosphate pathway; Bissett and Anderson, 1974). Recent studies on *S. lactis* and *S. cremoris* indicate that all genes coding for the initial uptake of lactose by PTS and the enzymes involved in the D-tagatose 6-phosphate pathway are plasmid-linked (Anderson and McKay, 1977; Crow et al., 1983). Lactose permease and β-galactosidase are cryptic in streptococci and are only functioning in strains lacking PTS. However, the rates of lactose fermentation and growth are significantly lower in such strains (Thomas, 1976; Thompson and Thomas, 1977).

As pointed out by Lawrence and Thomas (1979), "the PEP-dependent PTS for lactose uptake and phospho-β-galactosidase appear as prerequisites for rapid homolactic lactose fermentation by lactic streptococci".

Fermentation of pentoses

Pentoses are usually fermented by all heterofermentative lactic acid bacteria although there are some pentose-negative strains. As depicted in Fig. 3, pentoses are taken up by specific permeases and converted by appropriate enzymes to D-xylulose 5-phosphate which is fermented to lactate and acetate according to the lower half of the 6-phospho-gluconate pathway (cf. Gottschalk, 1979; cf. Doelle, 1975).

Only very few lactobacilli are able to grow on pentitols which are taken up by the action of a PEP-dependent PTS. The pentitol phosphates thus formed are oxidized by the appropriate dehydrogenases and isomerized to D-xylulose 5-phosphate (London and Chace, 1977, 1979), the common intermediate of pentose and glucose fermentation, via the 6-P-gluconate pathway. In contrast to the constitutive enzymes of glucose fermentation, the additional enzymes required for pentose fermentation are inducible by the respective pentoses. Although no detailed studies on the genetical basis of pentose fermentation in lactobacilli are available, one may assume that the respective enzymes are also coded in a regulatory unit (regulon) as is known for *Escherichia coli* (Sheppard and Englesberg, 1966, 1967; Irr and Englesberg, 1970; Katz, 1970; Greenblatt

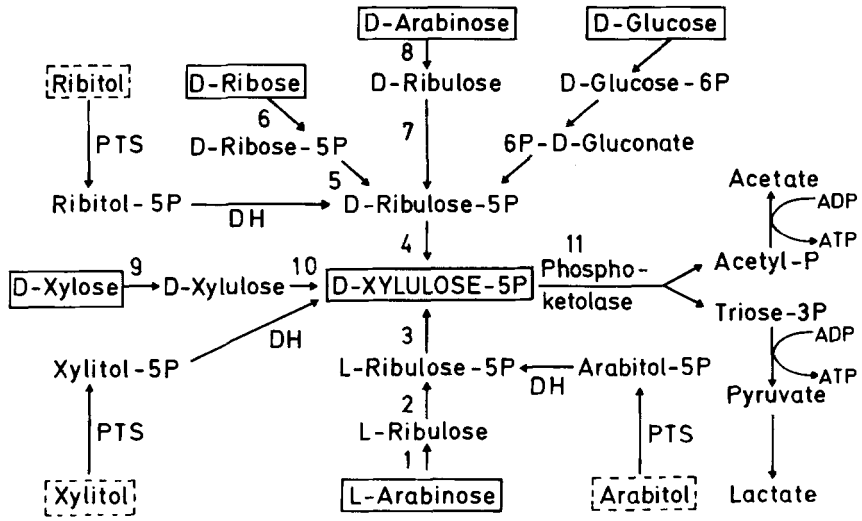

Fig. 3. Scheme of pentose and pentitol dissimilation in lactic acid bacteria.
1 = L-Arabinose ketol-isomerase; 2 = ATP: L-Ribulose 5-phosphotransferase; 3 = L-Ribulose 5-phosphate 4-epimerase; 4 = D-Ribulose 5-phosphate 3-epimerase; 5 = D-Ribose 5-phosphate ketol-isomerase; 6 = ATP: D-Ribose 5-phosphotransferase; 7 = ATP: D-Ribulose 5-phosphotransferase; 8 = D-Arabinose ketol-isomerase; 9 = D-Xylose ketol-isomerase; 10 = ATP: D-Xylulose 5-phosphotransferase; 11 = D-Xylulose 5-phosphate D-glyceraldehyde 3-phosphate lyase; DH = Dehydrogenase.

and Schleif, 1971; Zubay et al., 1971). This is indicated by studies on three groups of ethyl methane sulfonate (EMS)-induced arabinose-negative mutants of *Lb. cellobiosus* (H. Stetter, 1974). The representatives of these groups were permease-negative and defective with respect to only one enzyme typical of the L-arabinose pathway – either enzyme No. 1, 2, or 3, listed in Fig. 3 – whereas the other enzymes were still inducible by L-arabinose. On the other hand, none of several L-arabinose-negative wild types exhibit an inducible activity of any of these enzymes. Back mutations occurred at rates of 10^{-8} to 10^{-9}, but no L-arabinose-positive mutant was obtained by EMS-treatment of several L-arabinose-negative wild types. Hence, L-arabinose-negative wild types are not merely single-step defect mutants but may lack the whole arabinose operon.

The necessity of phosphoketolase for pentose fermentation would suggest that only heterofermentative lactic acid bacteria were able to ferment pentose. In fact, the strictly homofermentative so-called thermobacteria are unable to ferment any pentoses. (Contrasting reports are questionable. When the authors sent us strains claimed to be pentose-positive thermobacteria, we either could not confirm pentose fermentation or we were not able to confirm the designation as a thermobacterium.)

However, many strains of the so-called "streptobacteria", the streptococci

and the pediococci ferment pentoses readily, although they, too, exhibit homolactic fermentation of hexoses and contain a constitutive aldolase. In these cases phosphoketolase is an inducible enzyme with pentoses functioning as inducers. Upon induction, pentose fermentation proceeds via the same pathway as in heterofermentative organisms, resulting in equimolar amounts of lactate and acetate. Thus, streptobacteria, streptococci and pediococci are heterofermentative with respect to pentose fermentation. They are, therefore, designated as being facultatively homofermentative in Table 1.

Two other reports, hitherto neglected in the literature, indicate that some lactic acid bacteria may ferment even pentoses by a homolactic pathway. Fukui et al. (1957) report the formation of predominantly lactate (up to 95%) and small amounts of formate but not acetate from L-arabinose and D-ribose by *S. faecalis* and *S. glycerinaceus*. Barre (1978) reports the almost exclusive formation of lactate from L-arabinose and D-ribose by an unnamed thermophilic homofermentative lactobacillus isolated from fermenting grape must. Both authors concluded that pentose is dissimilated by an, as yet, unknown pathway which does not involve phosphoketolase. We investigated Barre's new isolates and confirmed his findings. We also found that *Sporolactobacillus inulinus* ferments D-ribose to lactate. However, this species is no longer considered as belonging to the lactic acid bacteria. Studies on 16S rRNA homology have shown that it is specifically related to members of the genus *Bacillus* (Stackebrandt and Woese, 1981; Stackebrandt et al., 1983). Bacilli do not possess phosphoketolase but dissimilate pentoses by the glycolytic pathway subsequent to the transformation of the pentose molecule to hexoses or trioses via transaldolase and transketolase reactions.

ALTERNATIVE FATES OF PYRUVATE

The technologically most important alternative fate of pyruvate is its conversion to acetoin and diacetyl (butter aroma) which proceeds via two different pathways as depicted in Fig. 4. While the formation of acetoin via acetolactate, followed by oxidation to diacetyl, was formerly supposed to be the only pathway of diacetyl synthesis, its direct synthesis from acetyl-CoA and activated acetaldehyde could be demonstrated later (Speckman and Collins, 1968, 1973). Both pathways occur in the same organism (Jönsson and Pettersson, 1977). Formation of acetoin and diacetyl is low, when hexoses are the only carbon sources but it is significant if additional pyruvate originating from the breakdown of organic acids, e.g. citrate (milk!), is available. The extent to which flavorous compounds, such as diacetyl and acetaldehyde or flavourless, more reduced compounds, such as acetoin, butyleneglycol or ethanol, are formed, depends on the oxidation-reduction balance of the system.

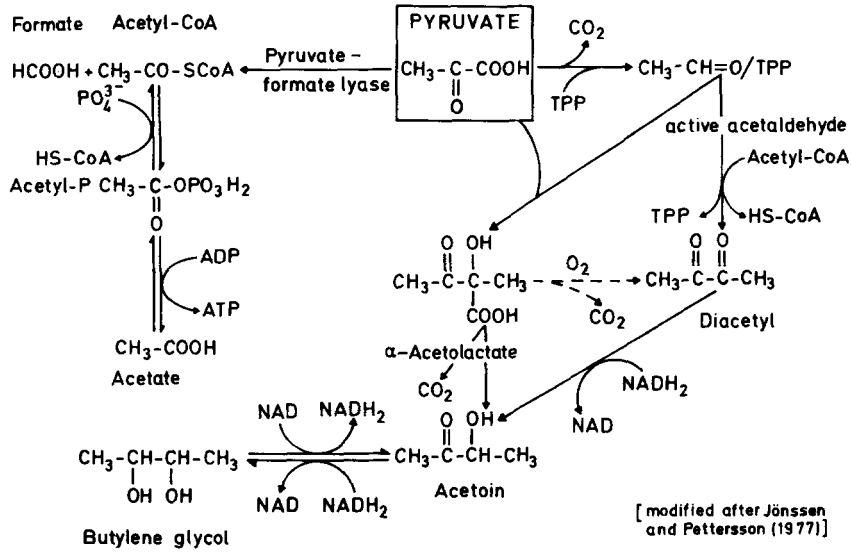

Fig. 4. Alternative pathways of anaerobic pyruvate dissimilation in lactic acid bacteria.

As further shown in Fig. 4, pyruvate can also be split to acetate and formate by pyruvate formate lyase as shown in the case of bifidobacteria (De Vries and Stouthamer, 1968; Lauer and Kandler, 1976), *Lb. casei* (De Vries et al., 1970; Lawrence and Thomas, 1979) and leuconostocs (Winter, 1974). The extent of the lyase reaction depends on the strain and the glucose concentration. Under conditions of glucose limitation, almost all intermediarily formed pyruvate may be cleaved. Thus, homolactic fermentation is converted to heterofermentation with acetate, ethanol and formate as end-products, and the heterolactic fermentation of bifidobacteria or leuconostocs resembles a mixed acid fermentation rather than a heterolactic one.

Although lactic acid bacteria contain neither cytochromes nor catalase – at least not in the absence of heme (cf. London, 1976) – they are able to carry out oxidations catalysed by flavin-containing oxidases and peroxidases or NAD-independent dehydrogenases which also contain flavin (Hager et al., 1954; Snoswell, 1959, 1963; Strittmatter, 1959*a, b*; Brown and VanDemark, 1968, London, 1968). Hence, they are able to oxidize at least part of the intermediarily formed pyruvate and even lactate, the normal end-product of lactic acid fermentation.

Two mechanisms for converting pyruvate to acetate and CO_2 without formate production, each yielding 1 mol ATP per mol pyruvate, have been reported for lactic acid bacteria. One requires lipoate and coenzyme A, involves acetyl-CoA and acetylphosphate (Fig. 5) as intermediates and has been found in *S. faecalis*

(O'Kane and Gunsalus, 1948; Gunsalus et al., 1952). In the other mechanism, reported for *Lb. delbrückii* (Hager et al., 1954) and *Lb. plantarum* (Dirar and Collins, 1973; Götz et al., 1980*b*) lipoate and coenzyme A are not required and acetylphosphate is directly formed during oxidation. Under limiting galactose concentration and under aerobic conditions as much as 93% of the hexose carbon was converted to acetate and CO_2 by *Lb. plantarum*. Thereby approximately 2 mol ATP per mol of hexose were gained (Dirar and Collins, 1973) in addition to the ATP formed under anaerobic conditions (Dirar and Collins, 1972). The enzymes, catalysing pyruvate oxidation have not been studied very intensively. While a pyruvate oxidase has been demonstrated in *Lb. plantarum* under aerobic conditions (Götz et al., 1980*a*), a pyruvate dehydrogenase seems more likely to be active in the anaerobic pyruvate dissimilation to acetate and CO_2, especially when acetyl-CoA is involved. Although the details of pyruvate dissimilation have not yet been fully elucidated the following picture may be drawn (Fig. 5): under anaerobic conditions some of the pyruvate may be oxidized by an NAD-dependent pyruvate dehydrogenase and, under liberation of CO_2, converted to acetate via acetyl-CoA and acetylphosphate.

In the absence of O_2, $NADH_2$, originating from glycolysis and pyruvate oxidation, has to be reoxidized by transfer to an acceptor present in the medium or formed in the cell, e.g. acetyl-CoA, thus yielding ethanol. However, no ethanol was found in strictly anaerobic fermentations of galactose with *Lb. plantarum* although equimolar amounts of lactate and acetate were formed (Dirar and Collins, 1973). Since no other reduced product was reported and no oxidation-reduction balance is given, the mechanism of $NADH_2$ reoxidation remains obscure.

It is easy to envisage how the oxidation-reduction balance will be under aerobic conditions. When pyruvate is oxidized by pyruvate reductase, $NADH_2$ may be reoxidized by the cooperation of $NADH_2$ oxidase and $NADH_2$ peroxidase. Both enzymes are known to be very active in lactic acid bacteria (Götz et al., 1980*b*). When pyruvate oxidase is involved, only $NADH_2$ peroxidase is required to reoxidize the surplus of $NADH_2$ originating from glycolysis with the equimolar amount of H_2O_2 obtained from pyruvate oxidation.

Oxidative dissimilation of lactate

Although lactate is the end-product of lactic acid fermentation it can be further metabolized to acetate and CO_2 under aerobic conditions by stereospecific NAD-independent, flavin-containing lactate dehydrogenases or lactate oxidases. Strittmatter (1959*a*) reported lactate oxidation by *Lb. casei* and *Lb. plantarum* in the presence of methyleneblue (MB), indicating the action of a lactate dehydrogenase, while London (1968) found in *S. faecium* lactate oxidation with O_2 as electron acceptor indicative of an oxidase. Among 23 lactobacilli tested

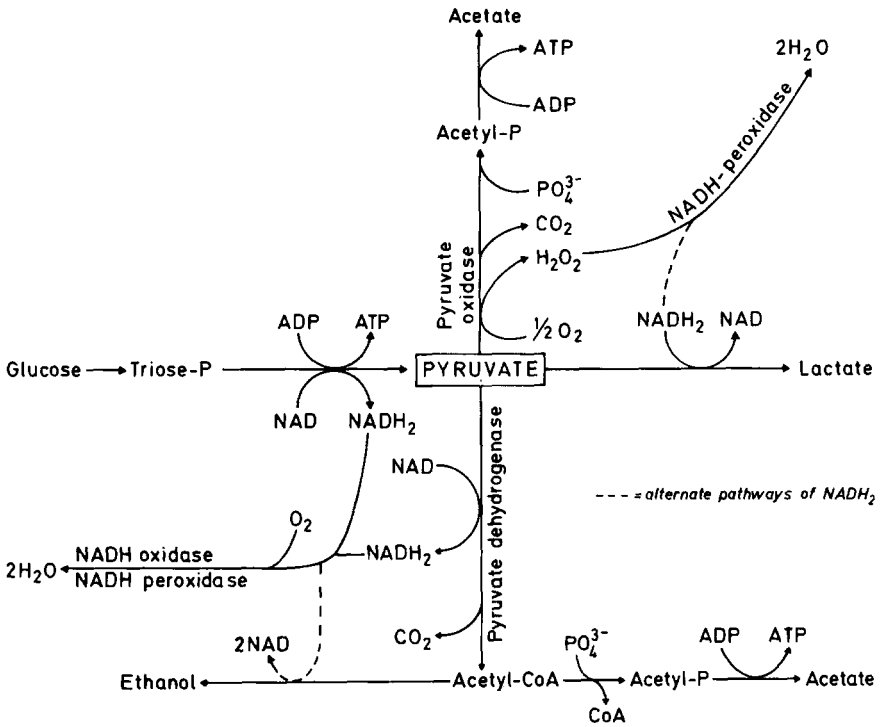

Fig. 5. Hypothetic scheme of oxidative pyruvate dissimilation.

in our laboratory (Höchst, 1979) 21 strains were found to oxidize lactate. They can be divided into two groups. One possesses a flavin-containing L-lactate oxidase using O_2 as electron acceptor and producing H_2O_2 (Fig. 6), the other requires MB to perform lactate oxidation at significant rates. In group I lactate oxidation with O_2 yields H_2O_2 and pyruvate. Both products accumulate to a certain extent during lactate dissimilation by washed cells and cell homogenates and lead to spontaneous oxidative splitting of pyruvate, which could be prevented by the addition of catalase. The in vivo dissimilation of pyruvate in group I remains to be further elucidated. Catalase also decreased the O_2 uptake by 50% as is expected if H_2O_2 is split. Group II exhibited only a very low rate of lactate oxidation to acetate and CO_2 which was increased tenfold by the addition of MB yielding H_2O_2 and pyruvate, the accumulation of which leads to spontaneous splitting of pyruvate. The addition of dichlorophenolindophenol (DCPIP) resulted in an accumulation of pyruvate and $DCPIP_{red}$. The in vivo electron carrier reacting with artificial redox substances in vitro and the mechanism of pyruvate-splitting following lactate oxidation remain to be elucidated.

The interesting question of whether aerobically grown *Lb. plantarum* contains superoxide dismutase to protect the organism from the deteriorating effect of

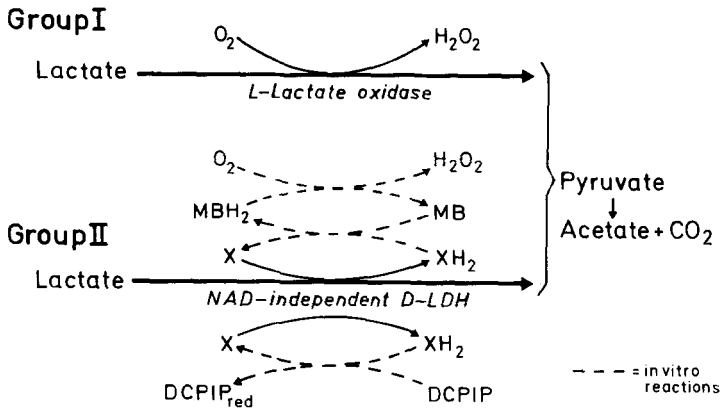

Fig. 6. Alternative mechanisms of lactate oxidation.
Group I = *Lb. curvatus, Lb. sake, Lb. acidophilus, Lb. bulgaricus, Lb. lactis;* Group II = *Lb. plantarum, Lb. casei, Lb. coryniformis; Streptococcus faecium.* (X = unknown electron carrier in vivo.)

O_2^- was recently studied very intensively. It was shown by Götz et al. (1980*a*) and confirmed by Archibald and Fridovich (1981), that *Lb. plantarum* does not contain superoxide dismutase. Instead, Mn^{2+} is responsible for the catalytic scavenging of O_2^-. The reaction of Mn^{2+} with O_2^- in the presence of pyrophosphate or phosphate is well known from experiments with chloroplasts (Lumsden and Hall, 1975; Kono et al., 1976) and model systems (Stein et al., 1979; Archibald and Fridovich, 1982; Götz and Lengfelder, 1983). The replacement of superoxide dismutase by Mn^{2+} as an O_2^- scavenger is a unique feature of lactic acid bacteria and the very high manganese requirement and content of lactobacilli may reflect this function (Archibald and Fridovich, 1981) rather than the manganese requirement of several enzymes, e.g. DNA-dependent RNA polymerase (Stetter and Kandler, 1973*b*; Stetter and Zillig, 1974), which are saturated at much lower concentrations.

Enzymatic basis for the occurrence of different isomers of lactate

Unlike the higher animals and plants which produce exclusively the L(+)-isomer, species of lactic acid bacteria produce either D(−)-or L(+)-lactate or even both isomers. The type of isomer formed is species- or even genus-specific and since Orla-Jensen (1919) has been used as a taxonomic marker. D(+)-Lactate is not readily utilized by man and animals; it has therefore been considered as the non-physiological one of the two isomers (Cori and Cori, 1929; Dunlop and Hammond, 1965; Krusch, 1978; Giesecke et al., 1981). The FAO/WHO-experts (1967) suggested limiting the daily intake of D(−)-lactate to 100 mg/kg body weight and attempts to favour the L(+)-isomer content in fermented food

are in progress (K. O. Stetter, 1974; Kunath and Kandler, 1980).

It is well known, that the particular isomers are formed by NAD-dependent lactate dehydrogenases (LDH) of the respective stereospecificity. When racemate is formed both D- and L-LDH are present, though often not with equal activity thus giving rise to an excess of one of the two isomers. In most species the ratio of the two isomers depends on the growth phase as exemplified in *Lb. acidophilus* (Lauer et al., 1980). In only three species of *Lactobacillus* does racemate result from the cooperation of an L-LDH and a lactate racemase. Lactate racemase was originally thought to be very widespread in lactic acid bacteria (Kitahara et al., 1957). Further studies confirmed the presence of lactate racemase only in *Lb. sake* and, additionally, demonstrated racemase in *Lb. curvatus* and *Lb. casei* subsp. *pseudoplantarum* (Stetter and Kandler, 1973*a*). Lactate racemase is an inducible enzyme with L(+)-lactate acting as an inducer. The induction depends on manganese (Stetter and Kandler, 1973*b*), which has been shown to be the divalent metal necessary for the DNA-dependent RNA polymerase of lactic acid bacteria (Stetter and Zillig, 1974), while the other bacteria require magnesium.

Together with fructose 1,6-bisphosphate, manganese is also an activator of the allosteric NAD-dependent L-LDH of *Lb. casei, Lb. curvatus* and *Lb. sake* (De Vries et al., 1970; Hensel et al., 1977) while all the allosteric L-LDH's of streptococci and bifidobacteria require only fructose 1,6-bisphosphate as effector. Detailed studies of L-LDH's have shown a considerable diversity not only between the enzymes of phylogenetically very distant organisms, but even within those of lactobacilli. However, they are all derived from the same ancestral gene as evidenced by the significant homology of the amino acid sequence of the substrate-binding region (Hensel et al., 1981; Mayr et al., 1982). In addition to NAD-dependent LDH, most lactic acid bacteria contain also NAD-independent LDH's. They are, however, not involved in lactate formation, but rather in lactate oxidation as mentioned above.

It is tempting to ask, whether the distinct diversification of the L-LDH's and the occurrence of all possible combinations of lactate isomers within the lactic acid bacteria are of any physiological or ecological advantage. Unfortunately, there is no reasonable answer. Physiologically, the modification to an allosteric LDH seems the most advantageous invention since it allows new regulatory functions. However, the species that do not possess regulated LDH's are growing just as well and do not seem to be less competitive.

The much more pronounced diversification, not only of the LDH's in lactic acid bacteria but in the prokaryotes as a whole compared to that in higher organisms, may merely reflect the much higher phylogenetic age of the prokaryotes (Kandler, 1981; Woese, 1982) as such and the rather early speciation within the lactic acid bacteria (Stackebrandt et al., 1983). The latter is clearly shown by the 16S rRNA relatedness (S_{AB} values; cf. Stackebrandt and Woese, 1981) among the species of lactic acid bacteria, which is much lower than among the aerobic bacilli or micrococci.

Concluding remarks

Carbohydrate metabolism in lactic acid bacteria has attracted the interest of physiologists and chemists since the very beginning of research in microbiology. It has received attention not only with respect to its biotechnological relevance but also because of the ease with which clear carbohydrate fermentation balances could be achieved which in their turn promised to lead to rapid progress in the elucidation of the fermentation mechanisms. It took, however, a full half century before in the 1950's we had sufficient bits of chemical and enzymological evidence to enable us to draw a detailed picture of the main pathways discussed at the beginning of this paper. The booming progress in biochemical knowledge of lactic acid fermentations in the 1950's was consolidated in the 1960's, and the 1970's brought us insight into the mechanisms of carbohydrate uptake in the cell, knowledge of the regulatory sites within the various pathways, and, last but not least, a first glimpse on the functioning of genetic coding and control in the carbohydrate metabolism of lactic acid bacteria. As a consequence, the 1980's will belong to the metabolic and genetic control of growth and fermentation and the 1990's will be ruled by the translation of the accumulated knowledge into biotechnological reality, such as the "construction" of optimized lactic acid bacteria by genetic engineering or the development of stabilized continuous fermentations by combined metabolic and genetic control.

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