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REVIEW

Microbial production of lactic acid

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Abstract Lactic acid is an important commodity chemical having a wide range of applications. Microbial production effectively competes with chemical synthesis methods because biochemical synthesis permits the generation of either one of the two enantiomers with high optical purity at high yield and titer, a result which is particularly beneficial for the production of poly(lactic acid) polymers having specific properties. The commercial viability of microbial lactic acid production relies on utilization of inexpensive carbon substrates derived from agricultural or waste resources. Therefore, optimal lactic acid formation requires an understanding and engineering of both the competing pathways involved in carbohydrate metabolism, as well as pathways leading to potential by-products which both affect product yield. Recent research leverages those biochemical pathways, while researchers also continue to seek strains with improved tolerance and ability to perform under desirable industrial conditions, for example, of pH and temperature.

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Introduction

Lactic acid is a three carbon α-hydroxycarboxylic acid with a single chiral center, which therefore exists as one of two stereoisomers, D-lactic acid and L-lactic acid. A weak acid with a pK_A of 3.86, lactic acid (lactate) has a wide range of applications in the food, cosmetic, agricultural and pharmaceutical industries (Hofvendahl and Hahn-Hägerdal 2000; Datta and Henry 2006; Okano et al. 2010). Lactate is also of interest for its potential chemical conversion into other high volume chemicals, such as hydrogenation to 1,2-propanediol or dehydration to acrylic acid (Datta and Henry 2006; Gao et al. 2011). Lactic acid is principally used as the starting material for poly(lactic acid) (PLA), a renewable and biodegradable polymer (Wehrenberg 1981; Kharas et al. 1994). PLA can be produced via the cyclic dimer lactide which allows for a controlled polymer molecular weight (Drumright et al. 2000). Several properties of PLA including crystallinity and thermal stability are moreover impacted by the relative fraction of the L- and D-isomers either in the polymer chain or in blends (Gruber et al. 1992, 1996; Lunt 1998; Blomqvist 2001; de Jong et al. 2001; Tsuji 2002; Tsuji and Fukui 2003). Lactic acid produced from petrochemicals, however,

results in a racemic mixture, whereas microorganisms can generate exclusively either L-lactic acid or D-lactic acid, depending on which lactate dehydrogenase (LDH) enzyme is expressed to convert pyruvate to lactate as the final biochemical step. Thus, a great advantage of the biological route is the generation of optically pure lactate which directly affects the subsequent polymer composition (Wehrenberg 1981; Blomqvist 2001; Tsuji 2002).

As a commodity chemical, lactic acid must be biologically produced from cheap starting materials derived from agricultural sources such as the carbohydrate glucose. The ultimate yield of lactic acid (i.e., g product generated per g substrate consumed) is determined both by the biochemical pathways in upper metabolism which convert the carbohydrate into metabolic intermediates and by pathways in lower metabolism which potentially generate undesirable by-products. Thus, research can broadly be categorized as affecting upper metabolism or lower metabolism.

Upper metabolism involved in lactic acid formation

Lactic Acid Bacteria (LAB) are classified as either *homofermentative* or *heterofermentative* based on the metabolism of glucose. Although the terms are typically applied to LAB, many other organisms that generate lactic acid share features of the same pathways and can be considered homofermentative or heterofermentative. As the name implies, a homofermentative lactic acid process is one which potentially generates lactate alone. In this case, glucose is metabolized through the Embden–Meyerhof–Parnas (EMP) pathway (Fig. 1a, b). Key enzymes in the EMP pathway include glucose-6P isomerase (EC 5.3.1.9), 6-phosphofructokinase (EC. 2.7.1.11), and fructose-bisphosphate aldolase (EC 4.2.1.13). Conversion of glucose exclusively through the EMP pathway leads to a maximum theoretical yield of 2 mol lactate per mol glucose:

$$\text{Glucose} + 2\text{P} + 2\text{ADP} \rightarrow 2\text{Lactate} + 2\text{ATP} + 2\text{H}_2\text{O} \quad (1)$$

In contrast, a heterofermentative lactic acid process is one in which a significant fraction of the glucose is metabolized via the phosphoketolase (PK) pathway (Fig. 1a): from six-carbon gluconate-6P, the enzyme 6-phosphogluconate dehydrogenase (EC 1.1.1.44) cleaves of a CO_2 to form ribulose-5P, and then phosphoketolase (EC 4.1.2.9) generates glyceraldehyde-P and acetate-P. Once metabolism has committed to acetate-P formation, this 2-carbon metabolite is not readily converted "back" to a 3-carbon compound, constraining this pathway to a maximum theoretical yield of 1 mol lactate per mol glucose. If the acetate-P formed is hydrolyzed to acetate by acetate kinase, then the stoichiometric conversion of glucose through the PK pathway becomes:

$$\text{Glucose} + 2\text{NAD(P)} + \text{P}_i + 2\text{ADP} \rightarrow \text{Lactate} + \text{Acetate} + \text{CO}_2 + 2\text{NAD(P)H} + 2\text{ATP} + \text{H}_2\text{O} \quad (2)$$

Equation 2 is incomplete since NAD(P)H must be oxidized to complete a redox balance. Depending on enzymes present, NAD(P) is regenerated as a result of the reduction of O_2 or the reduction of other metabolites.

A third pathway found in upper metabolism that influences lactic acid formation is the pentose phosphate (PP) pathway (Fig. 1b). In this pathway, the 5-carbon ribulose-5P formed through 6-phosphogluconate dehydrogenase partitions between xylulose-5P and ribose-5P (two 5-carbon compounds), that are reversibly shuttled through sedoheptulose-7P and glyceraldehyde-3P (7-carbon + 3-carbon), and erythrose-4P and fructose-6P (4-carbon + 6-carbon) by the actions of the key enzymes transaldolase (EC 2.2.1.2) and transketolase (EC 2.2.1.1). The EMP pathway mediates the conversion of the fructose-6P generated by the PP pathway ultimately into glyceraldehyde-3P. If glucose is metabolized exclusively through the PP pathway to glyceraldehyde-3P and fructose-6P and the latter is converted to glyceraldehyde-3P via the EMP pathway, then the maximum theoretical lactate yield is 1.67 mol per mol glucose:

$$3\text{Glucose} + 6\text{NAD(P)} + 5\text{P}_i + 5\text{ADP} \rightarrow 5\text{Lactate} + 3\text{CO}_2 + 6\text{NAD(P)H} + 5\text{ATP} + 2\text{H}_2\text{O} \quad (3)$$

Equation 3 is also incomplete, however, since additional reduced compounds (such as H_2O) must be generated to satisfy the redox balance.

Many microorganisms are able to metabolize pentoses such as D-xylose or L-arabinose, leading to

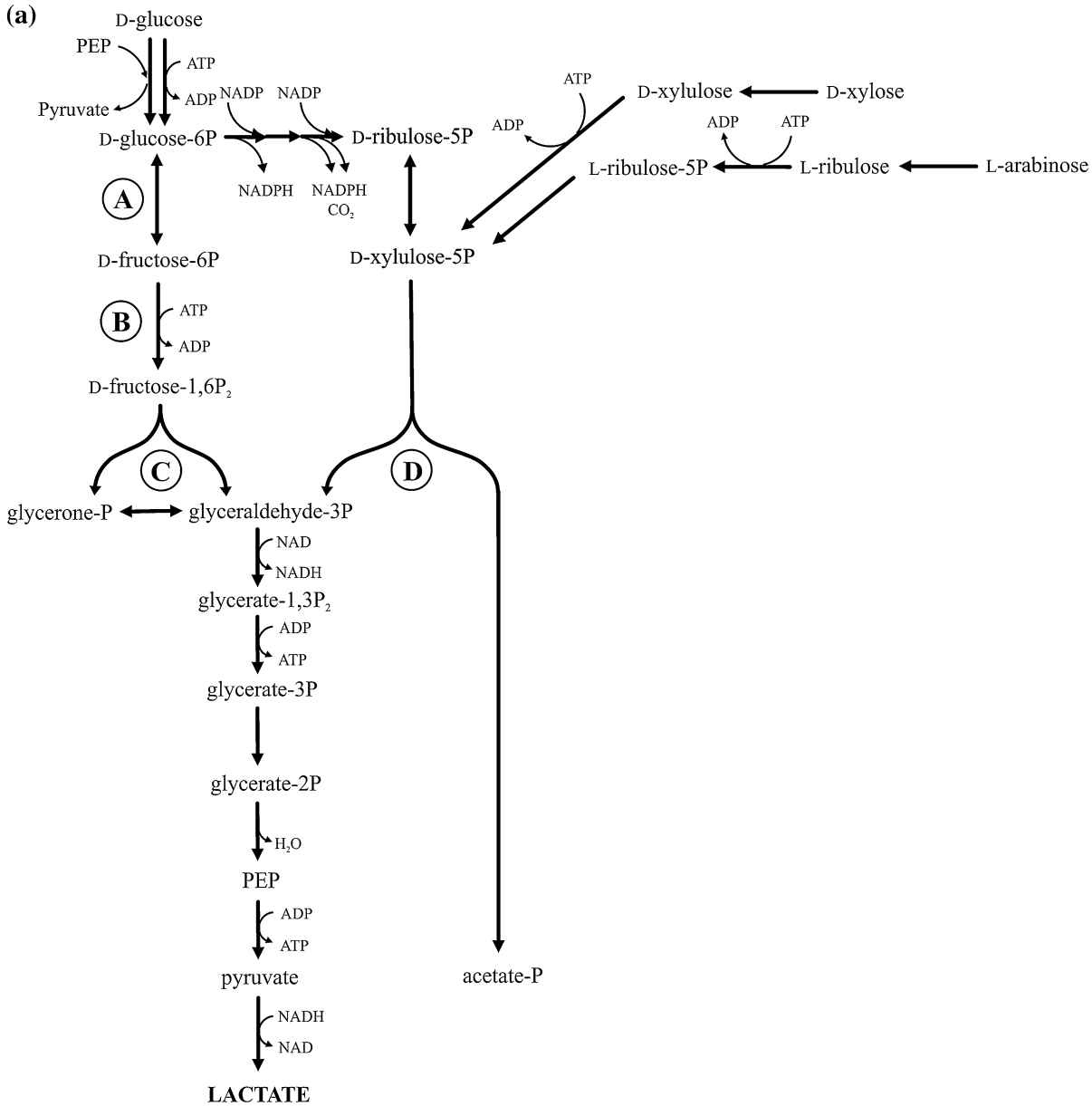

Fig. 1 Upper metabolism of microbes converting to simple sugars into lactic acid (lactate) and acetyl phosphate (acetate-P). **a** The Embden–Meyerhof–Parnas (EMP) pathway and the phosphoketolase (PK) pathway. Key enzymes in the EMP pathway include (*A*) glucose-6P isomerase (EC 5.3.1.9) (*B*) 6-phosphofructokinase (EC. 2.7.1.11) and (*C*) fructose-bisphosphate aldolase (EC 4.2.1.13). The key enzyme in the PK pathway is (*D*) phosphoketolase (EC 4.1.2.9). **b** the Embden-Meyerhof-Parnas (EMP) pathway and the pentose phosphate (PP) pathway. Key enzymes in the EMP pathway are as in **a**. The key enzymes in the PP pathway are: (*E*) transaldolase (EC 2.2.1.2) and (F) transketolase (EC 2.2.1.1). In each case, acetate-P is not an end-product, but is converted into acetate and/or ethanol (see Fig. 2). Not every lactic acid-generating microorganism expresses all the enzymes shown, and some microorganisms deviate from this general scheme

lactate formation. Of course, these organisms must have an appropriate transport mechanism and enzymes to enter one of the major pathways described above. Because the PK pathway partitions 5-carbon compounds directly into glyceraldehyde-3P and acetate-P, the conversion of pentoses through this

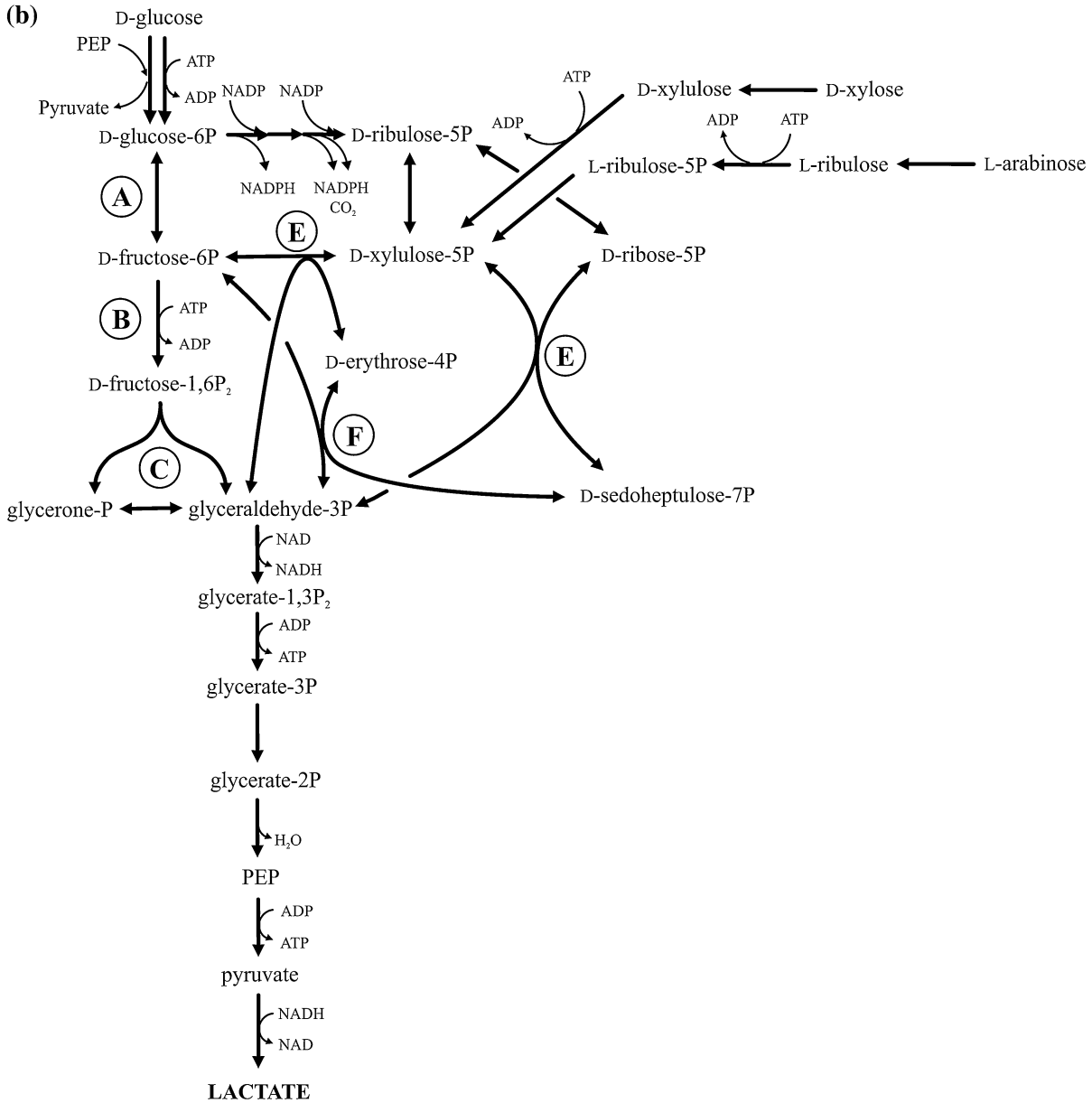

Fig. 1 continued

pathway results in a maximum yield of 1 mol lactate per mol pentose. Using xylose as an example pentose:

$$\text{Xylose} + 2\text{ADP} + 2\text{P}_i \rightarrow \text{Lactate} + \text{Acetate} + 2\text{ATP} + 2\text{H}_2\text{O} \quad (4)$$

Equation 4 is written with the assumptions that the product of the PK pathway, acetate-P, is converted entirely to acetate, and that xylose transport into the cell is by facilitated diffusion (and does not consume ATP).

Alternatively, pentoses such as D-xylose or L-arabinose can be metabolized by the PP pathway. Because this pathway does not commit the cells to acetate-P formation, the maximum theoretical yield of lactate in this case is 1.67 mol/mol pentose:

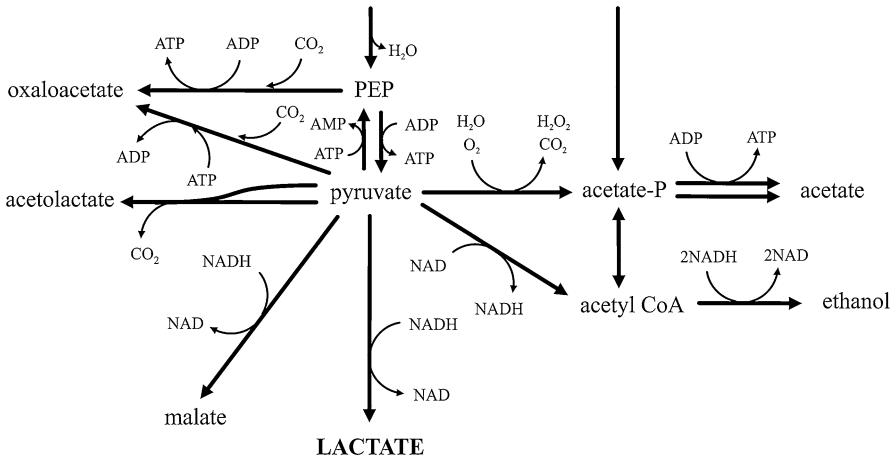

Fig. 2 Lower metabolism of microbes showing the conversion of phosphoenol pyruvate (PEP) and acetyl phosphate (acetate-P) into lactate and other by-products. Not every lactic acid-generating microorganism expresses all the enzymes shown, and some microorganisms deviate from this general scheme

$$3\text{Xylose} + 2P_i + 2\text{ADP} \rightarrow 5\text{Lactate} + 2\text{ATP} \quad (5)$$

Importantly, because Eq. 5 is redox balanced, the conversion of pentoses to lactate can proceed like a homofermentative process if the PP pathway is the exclusive metabolic pathway used for the conversion. However, the PK pathway generates 2 ATP/mol xylose consumed (Eq. 4) while the PP pathway generates 0.67 ATP/mol xylose consumed (Eq. 5).

Many microbial species have two or three of these pathways, and thus the resulting intermediate lactate yield is determined by which pathway predominates under the particular growth conditions. Based on Eqs. 1–5, the theoretical lactate yield from glucose resulting from the EMP, PP or PK pathways is 2, 1.67 or 1 mol, respectively. The lactate yield from xylose resulting from the PP or phosphoketolase pathway is 1.67 or 1 mol, respectively. Thus, the greatest lactate yield occurs for cells which metabolize glucose exclusively through the EMP pathway and which metabolize pentoses exclusively through the PP pathway.

Conversion of pentoses to lactic acid

Since different pathways in upper metabolism affect the stoichiometry of lactate generation, lactic acid yield can be improved merely by modifying an organism to favor a higher yielding pathway. For example, for the conversion of arabinose, the endogenous phosphoketolase gene in *Lactobacillus plantarum* was substituted with the transketolase gene from *Lactococcus lactis*, thereby shifting the PK pathway to the PP pathway (Okano et al. 2009a). With this improved pathway, 50 g arabinose l^{-1} was converted to 38.6 g lactic acid l^{-1} (yield = 1.54 mol/mol = 0.77 g/g) in 27 h. Although *Lb. plantarum* cannot utilize xylose, the same strategy of removing the PK pathway and introducing the PP pathway allowed a strain which also expressed two xylose-assimilation genes to generate 41.2 g lactic acid l^{-1} from 50 g xylose l^{-1} (yield = 1.65 mol/mol = 0.82 g/g) in 60 h (Okano et al. 2009b). In both these studies with *Lb. plantarum*, using an *ldhL* mutant led predominantly to the D-lactate isomer. Introducing the *Lactobacillus pentosus* xylose-assimilating operon *xylAB* into *Lb. plantarum ldhL* with additional knockouts in two phosphoketolase genes and a chromosomally integrated transketolase gene (*tkt*) allowed the strain to generate 74 g D-lactic acid l^{-1} from a mixture of 25 g xylose l^{-1} and 75 g glucose l^{-1} in 36 h without catabolite repression (Yoshida et al. 2011).

An organism which is able to use xylose, *L. lactis* IO-1 uses the PP pathway, at least partly, because the microbe naturally attains a yield greater than 1 mol lactate/mol glucose (Tanaka et al. 2002). Merely increasing the culture xylose concentration increases the lactate yield. Subsequent sequencing showed that *Lc. lactis* IO-1 contains transketolase but no transaldolase, and thus is thought to use 6-phosphofructokinase and fructose bisphosphate aldolase as alternate enzymes to complete the higher yielding PP pathway (Kato et al. 2012). Furthermore, the strain exclusively produces L-lactic acid, as genes coding D-lactate dehydrogenase (D-LDH) and lactate racemase are absent (Shimizu-Kadota et al. 2013). As an alternative to maintaining a high xylose concentration, the native PK pathway can be eliminated by deleting the

phosphoketolase gene (*ptk*) in *Lc. lactis* IL1403 (Shinkawa et al. 2011). A consequence of this knockout was a reduced rate of lactate formation, necessitating an additional copy of the transketolase gene (*tkt*) to restore the rate, and suggesting that lactate formation in *Lc. lactis* is limited by the flux through the PP pathway.

Conversion of sucrose to lactic acid

An *E. coli* engineered to accumulate D-lactic acid from sucrose was constructed by a knockout in the sucrose repressor gene (*cscR*), leading to 85 g D-lactic acid l^{-1} in 84 h with a yield of 0.85 g/g (Wang et al. 2012). A strain engineered to accumulate L-lactic acid, also evolved for improved growth on sucrose, resulted in the generation of 97 g L-lactic acid l^{-1} with a yield of 0.9 g/g in a defined medium containing sucrose (Wang et al. 2013). This strain also converts sugarcane molasses and corn steep liquor to 75 g L-lactic acid l^{-1} with a yield of 0.85 g/g (Wang et al. 2013).

The filamentous fungus *Rhizopus oryzae* has long been studied for lactic acid production (Zhou et al. 1999), and the microbe generates L-lactic acid from glucose, sucrose and starch (Zhang et al. 2007). Lactic acid production occurs only during growing conditions (Maas et al. 2008).

Conversion of lignocellulosic hydrolysates to lactic acid

Many agricultural substrates are composed of complex carbohydrates which must be hydrolyzed into their constituent sugars ("saccharification") prior to being converted into any biochemical product such as lactic acid ("fermentation"). There are several strategies to carry out this overall process. The conventional process for starch materials involves a saccharification step with high temperature gelatinization, followed by a second, separate fermentation step by appropriate organisms. Similarly, lignocellulosic materials may be chemically hydrolyzed, for example with dilute acid, prior to their fermentation.

A significant amount of research over many years has focused on hydrolyzing various lignocellulosic materials chemically, followed by the fermentation of the resulting hydrolysate into useful products, and one product studied has been lactic acid. For example, corncobs hydrolyzed with dilute sulfuric acid yielded a hydrolysate of 26.5 g xylose l^{-1}, 2.4 g glucose l^{-1}, 3.2 g arabinose l^{-1} and 3.5 g acetic acid l^{-1} which, after vacuum evaporation, was converted to 80 g lactic acid l^{-1} with a yield of about 80 % based on total sugar using a *R. oryzae* adapted to the hydrolysate (Bai et al. 2008). Similarly, *Bacillus* generated 55 g L-lactic acid l^{-1} from acid-hydrolyzed sugar cane bagasse supplemented with salts and corn steep liquor (Patel et al. 2004).

In most lignocellulosic materials, the hemicellulose fraction is readily hydrolyzed, for example with hot water. Such hydrolysates contain predominantly xylose, but often significant fractions of other sugars, and this mixture is an attractive source of carbohydrates for lactic acid. The moderately thermophilic *Bacillus coagulans* MXL-9 (described in detail below), has been used to ferment a several hot water hydrolysates. For example, larch extracts composed of 45.8 g total sugars l^{-1} was converted to 40.5 g lactic acid l^{-1} after 50 h (Walton et al. 2010). These cells consumed the glucose and mannose first, after which the remaining sugars were metabolized.

Guo et al. (2010a) isolated LAB strains whichthat metabolized both hexoses and pentoses found in lignocellulosic hydrolysate. Using a corncob hydrolysate, *Lactobacillus brevis* S3F4 generated 39 g lactic acid l^{-1} from a mixture containing about 57 g mixed sugars l^{-1}. Importantly, the *Lb. brevis* metabolized glucose and xylose simultaneously and showed no growth inhibition or reduced product formation in the presence of 10 mM furfural and ferulic acid. Guo et al. (2010b) solated over 100 strains of *R. oryzae* and examined them for lactic acid production. One strain (GY18) converted the sugar mixture found in corncorb hydrolysate (233 g xylose and 39 g glucose per kg corncob) to lactic acid, although the microbe consumed xylose only after glucose was exhausted.

Simultaneous saccharification and fermentation for lactic acid formation

Another approach to sequential saccharification then fermentation involves "simultaneous saccharification and fermentation" (SSF), which increases volumetric productivity by combining otherwise sequential processes. This strategy can be accomplished in several ways: (1) saccharifying enzymes and a fermenting microbe are added simultaneously to the substrates,

(2) a saccharifying microbe and a fermenting microbe are added simultaneously to the substrates, and (3) a single microbe is used which is able itself to saccharify the substrate and ferment the intermediate sugars.

Lactic acid may also be produced using SSF. This topic has been reviewed (John et al. 2009), and it continues to be a fruitful area of research. For example, using 275 g starch-containing cassava powder l^{-1} with 20 U glucoamylase l^{-1}, *Lactobacillus rhamnosus* generated 175 g L-lactate l^{-1} in 96 h (Wang et al. 2010a). Similarly, *Lactobacillus lactis* converted 100 g cellobiose l^{-1} into about 80 g lactic acid l^{-1} in 48 h (Singhvi et al. 2010). Addition of cellulase into an SSF resulted in 73 g lactic acid l^{-1} from 100 g sugarcane bagasse-derived cellulose l^{-1} after 48 h (Singhvi et al. 2010). Similarly, a *Lactobacillus delbrueckii* which could utilize cellobiose converted 100 g pure cellobiose l^{-1} into 90 g lactic acid l^{-1} in less than 40 h (Adsul et al. 2007), and, with the addition of cellulase, 80 g cellulose l^{-1} into 67 g lactic acid l^{-1} in about 80 h (Adsul et al., 2006). The strain converted cellotriose and higher cellooligosaccharides, too, though at lower specific rates. Thermotolerant *Bacillus* strains described below are particularly suited to SSF because they were specifically isolated for their ability to generate lactic acid at 50 °C or above. For example, *B. coagulans* 36D1 generated 80 g lactic acid l^{-1} from 100 g cellulose l^{-1} during the course of 264 h when supplemented with cellulase (Ou et al. 2011).

Waste materials are also common substrates for the production of lactic acid using SSF. For example, cardboard subject to enzymatic hydrolysis is a source for lactic acid production by *Lactobacillus coryneformis* ssp. *torquens* (Yáñez et al. 2005). In a fed-batch process, 23.4 g lactic acid l^{-1} was generated at a 0.51 g/g yield from glucose at a volumetric productivity of 0.48 g l^{-1} h^{-1}. Using the same strain, D-lactic acid production from filter paper was also studied in an SSF. This process showed better yield when cellobiase was added externally, reaching about 25 g lactic acid l^{-1} in 48 h with a yield of 0.72 g lactic acid/g cellulose (Yáñez et al. 2003). The improvement in yield in cellulose compared with glucose as a substrate suggests that the slow release of glucose favors lactic acid formation over biomass production. D-Lactic acid production has also been studied from defatted rice bran which was not subject to sterilization (Tanaka et al. 2006). Because the indigenous bacteria produced a racemic mixture of lactic acid, the pH was maintained at 5 to ensure only D-lactic acid production by the intended *Lb. delbrueckii*. About 30 g lactate l^{-1} at a yield of 0.28 g lactic acid/g rice bran or 0.78 g lactic acid/g soluble sugars was achieved. Recycled paper sludge generated 73 g lactic acid l^{-1} using *Lb. rhamnosus* in a culture supplemented with cellulolytic and xylanolytic enzymes (Marques et al. 2008).

A challenge in SSF is matching the optimal conditions for enzymatic saccharification with the optimal conditions for microbial conversion (e.g., temperature, pH). Thus, in the case of lactic acid accumulation via SSF, many researchers have sought to increase the tolerance to temperature and pH of the lactic acid-producing microbes (described below). John and Nampoothiri (2008) applied classical mutagenesis using nitrous oxide to *Lb. delbrueckii* to obtain two mutants, which in SSF of cassava bagasse starch hydrolysate, generated lactate at a yield > 90 % while the wild-type generated lactate at a yield of about 50 %.

Mixed cultures for lactic acid formation

Several studies have proposed the use of mixed cultures of microbes, wherein each microbe selected for the microbial consortium accomplishes one specific conversion. Such an approach can address one of several specific objectives, but in each case the two or more organisms comprising the culture must be compatible and have similar nutritional and environmental requirements.

One application of mixed cultures is based on the concept that some microbes very effectively convert glucose into lactic acid, while others may be effective at the conversion of pentoses into lactic acid. For example, a homofermentative strain will attain a higher yield from glucose because it relies on the EMP pathway, while a heterofermentative LAB will generate more ATP from pentoses through the PK pathway and thus can generate lactate with high productivity though some yield is sacrificed. Based on this rationale, a mixed culture of *Lb. rhamnosus* (homofermenter) and *Lb. brevis* (heterofermenter) has been studied. This culture will generate lactic acid from a xylose/glucose mixture or from a corn stover hydrolysate to which cellulase was added (Cui et al. 2011). Using two strains led to higher overall lactate yield and

productivity (0.7 g/g and 0.59 g l^{-1} h^{-1}) compared with using only the homofermenter (0.59 g/g and 0.49 g l^{-1} h^{-1}). The selection of strains is important for the efficient conversion of sugar mixtures.

Another application of mixed cultures is accomplishing a two-organism SSF: one microbe saccharifies the biomass while a second ferments the hydrolysate. For example, *Lactobacillus* ssp. and *Aspergillus niger* converted Jerusalem artichoke tubers into L-lactic acid (Ge et al. 2009). In this case, *A. niger* generated inulinase and invertase to hydrolyze the inulin to glucose, and *Lactobacillus* inoculation was delayed 12 h to allow for some glucose accumulation. Interestingly, the presence of *Lactobacillus* increased enzyme production by *A. niger*, presumably because of the removal of glucose from the culture. The system achieved approx. 120 g L-lactic acid l^{-1} with a yield of 0.945 g/g in a repeated-batch process.

A third and different application of mixed cultures involves using a consortium of engineered strains to convert sugar mixtures into lactic acid (Eiteman et al. 2009). For example, for the conversion of xylose and glucose, the strategy uses two strains: one unable to consume glucose, and one unable to consume xylose. When both strains were conferred with additional knockouts for lactate generation, each member of the mixture carried out the one conversion. Such a system adapts to a changing xylose/glucose composition by changing the relative population of each strain (Eiteman et al. 2008), and thus very effectively converts mixed sugar streams into products such as lactic acid (Eiteman et al. 2009).

Lower metabolism involved in lactic acid formation

One common challenge for the production of lactic acid is that numerous by-products can be generated, depending on the microbe used and the pathways that microbe possesses. Different LAB, particularly, express several enzymes which compete with LDH for the utilization of pyruvate. The LDHs present in an organism and their catalytic efficiency also determine the relative abundance of the two isomers in the product (Zheng et al. 2012). Furthermore, several paralogs show some L-LDH or D-LDH activity and reduce the optical purity of lactic acid (Rico et al. 2008). Figure 2 shows enzymes found in lower metabolism of several LAB associated with pyruvate and acetate-P, the products of the EMP, PP pathways and PK pathways. Different organisms have other competing pathways, the importance of which also depends on the starting carbon source used. Reducing by-product formation has been a consistent theme in studying lactic acid production by many bacteria and fungi.

Decrease of by-products formed from glucose

As a model organism which is easy to manipulate genetically, *E. coli* accumulates significant lactic acid from glucose by simply knocking out the *pflB* gene coding pyruvate formate lyase (Dien et al. 2001; Dien et al. 2002). Though *E. coli* predominantly generates the D-lactate isomer, it may readily be turned into an L-lactate producer by knocking out the native gene coding D-LDH (*ldhA*) and a gene coding for an L-LDH (Chang et al. 1999; Dien et al. 2002; Zhou et al. 2004). Often a "two-phase" process is implemented to accumulate lactic acid, whereby an aerobic growth phase is decoupled from a subsequent anaerobic or microaerobic phase leading to lactate production. A two-phase approach is indeed necessitated by the knockout of the *pflB* gene, or any other gene which curtails anaerobic cell growth. Advantages of a two-phase process are higher yields because no carbon is converted to biomass during the non-growth production phase, and greater tolerance to environmental conditions during lactate accumulation because cell growth is not actually required.

Many studies have focused on this strategy. For example, a *pflB* knockout overexpressing the *Streptococcus bovis ldh* gene generated 73 g L-lactic acid l^{-1} on a complex medium at a yield of 0.93 g/g (Dien et al. 2001). A strain with knockouts in pyruvate formate lyase, fumarate reductase (*frdBC*), alcohol dehydrogenase (*adhE*) and acetate kinase (*ackA*) grown in defined medium generated 48 g lactate l^{-1} of 99 % optical purity close to the theoretical maximum yield of 1 g/g (Zhou et al. 2003), and then replacing the chromosomal *ldhA* with an L-LDH gene led to 50 g L-lactate l^{-1} at a yield of 0.95 g/g (Zhou et al. 2004). In an acetate-auxotroph having knockouts in pyruvate dehydrogenase (*aceEF*), pyruvate oxidase (*poxB*), PEP synthase (*pps*) and pyruvate formate lyase, all which compete with LDH for pyruvate, *E. coli*

generated over 90 g lactate l^{-1} with a yield of 0.95 g/g with succinate as the single by-product (Zhu et al. 2007). Additionally knocking out *frdABCD* coding fumarate reductase, and ensuring that the co-substrate acetate was absent at the time the anaerobic phase commences led to 138 g lactate l^{-1} in defined medium with a yield of 0.99 g/g and less than 1 % by-products (Zhu et al. 2007). A similar set of knockouts has been scaled up to 3,000 liters and demonstrated the benefit of $Ca(OH)_2$ as the neutralizing agent (Liu et al. 2014). Other combinations of knockouts which decrease by-product formation have also been studied. For example, a phosphotransferase (*pta*) and PEP carboxylase (*ppc*) mutant generated 62 g D-lactate l^{-1} on a complex medium with a yield of 0.9 g/g (Chang et al. 1999), while knocking out the native D-LDH and expressing L-LDH from *Lactobacilllus casei* generated 45 g L-lactate l^{-1} with a yield of 0.7 g/g (Chang et al. 1999).

Corynebacterium glutamicum, used commercially for amino acid production, has recently been examined for lactic acid production. With a native L-LDH, *C. glutamicum* needs a knockout in this L-LDH and overexpression of D-LDH from *Lb. delbrueckii* to accumulate D-lactate from glucose. After aerobic cell growth, centrifuged, washed and resuspended cells (at 60 g l^{-1}) with repeated glucose addition accumulated 120 g D-lactate l^{-1} in 30 h in a high density resting cell approach (Okino et al. 2008). The system also accumulated 17 g succinate l^{-1} and less acetate. *Klebsiella* ssp. have also been examined for the production of lactic acid from glucose. *Klebsiella oxytoca* with knockouts in phosphotransacetylase, acetate kinase and alcohol dehydrogenase generated 13 g D-lactic acid l^{-1} from 20 g glucose l^{-1}, and also generated 24 g D-lactic acid l^{-1} from sugarcane molasses in 96 h (Sangproo et al. 2012).

There has long been an interest in using yeast, particularly *Saccharomyces cerevisiae*, for the production of lactic acid from glucose (Dequin and Barre 1994; Porro et al. 1995). The motivation is that *S. cerevisiae* tolerates a low pH of about 3.5 and is efficient at the conversion of glucose to ethanol through the EMP pathway at nearly theoretical yield. Therefore, although *S. cerevisiae* is naturally an ethanol producer, the microbe might similarly be able to accumulate the redox-neutral product, lactic acid, if ethanol formation were to be prevented. For example, a knockout in one of the pyruvate decarboxylase genes involved in ethanol generation (*PDC1*) and overexpressing the bovine *ldh* gene (via a plasmid) led to a lactate yield of 0.20 g/g and an ethanol yield of 0.26 g/g, though cell growth was repressed (Adachi et al. 1998). More recently, *PDC1* was similarly knocked out and two copies of the *ldh* gene from *Leuconostoc mesenteroides* integrated onto the *S. cerevisiae* chromosome (Ishida et al. 2006a). From 100 g glucose l^{-1}, this yeast strain generated about 62 g D-lactate l^{-1} when the pH was controlled and about 53 g D-lactate l^{-1} without pH control (final pH was 2.8). Knocking out both *PDC1* and *PDC5* and integrating two copies of the bovine *ldh* gene led to over 82 g L-lactate l^{-1} in 200 h at a yield of 0.815 g/g glucose (Ishida et al. 2006b). More recently, strains with single gene disruptions were screened to find strains with improved resistance to L-lactic acid at a pH of 2.6 (Suzuki et al. 2013). Overexpressing L-LDH in a *PDC1* knockout strain with four additional gene disruptions (*DSE2 SCW11 EOF3 SED1*) led to 48 g L-lactic acid l^{-1} compared to 38 g l^{-1} in the strain overexpressing the L-LDH in a *PDC1* knockout. Each of these recent studies used a medium containing 10 g yeast extract l^{-1} and 20 g peptone l^{-1}.

Saccharomyces cerevisiae is not the only yeast used for generating lactic acid from glucose, but by-products must invariably be eliminated and lactic acid formation enhanced. *Kluyveromyces marxianus* strains were constructed with the *Lactobacillus helveticus* L-LDH gene using phosphoglycerate kinase and pyruvate decarboxylase promoters at various points in the chromosome (Hause et al. 2009). With $CaCO_3$ added for pH control and deleting the native single copy of pyruvate decarboxylase (*PDC1*), *K. marxianus* generated about 99 g L-lactate l^{-1} (> 99 % enantiomerically pure) from 104 g glucose l^{-1} in 48 h without any ethanol production. This strain also generated 9.1 g lactate l^{-1} from 10.8 g glucose l^{-1} after 72 h without pH control, which led to a final pH of about 3. Similarly, *Candida boidinii* expressing codon-optimized bovine L-LDH using the *PDC1* promoter in a *PDC1* knockout resulted in about 86 g lactate l^{-1} in 48 h at a yield of 1 g/g glucose (Osawa et al. 2009). By knocking out *PDC1* and introducing two L-LDH genes derived from *Bos taurus* under the control of the *PDC1* promoter, *C. boidinii* was able to generate over 100 g L-lactic acid l^{-1} in 33 h (Ikushima et al. 2009).

A series of patents have been issued for the production of lactic acid in yeast. For example, with

a goal of producing lactic acid at a low pH so that base consumption is minimized and the acid form (i.e., lactic acid) predominates, several yeasts with knockouts in the pyruvate dehydrogenase complex and pyruvate decarboxylase were transformed with one or more copies of the LDH gene and a lactate transporter (Porro et al. 2006). The researchers noted that a higher concentration and productivity of lactic acid can be attained in a medium having a low concentration of Mg^{2+} or Zn^{2+} ions. Lactic acid will also accumulate using yeast variants which have temperature-sensitive alcohol dehydrogenase or pyruvate decarboxylase and which express human- or frog-LDH (Sawaii et al. 2011).

Issatachenkia orientalis has been developed for lactic acid production because this yeast tolerates a pH below 3 (Suominen et al. 2012; Miller et al. 2012). With knockouts in native pyruvate decarboxylase and lactate:ferricytochrome *c* oxidoreductase genes and overexpressing one or more copies of L-LDH, this yeast generated 60 g lactate l^{-1} at a yield of 0.6 g/g under microaerobic conditions.

Decrease of by-products from pentoses

Most of the same knockouts involved in the conversion of glucose to lactic acid apply also when pentoses are the substrates. For example, *E. coli* with essentially the same knockouts as described above to exclude by-products, as well as containing the chromosomally-integrated *Pediococcus acidilactici* L-LDH gene (*ldhL*), generated 42.9 g L-lactic acid l^{-1} from xylose under anaerobic conditions in about 120 h in a defined medium (Zhao et al. 2013). *Candida utilis* expressing xylitol dehydrogenase, xylulokinase and a xylose reductase mutated to favor NAD over $NADP^+$ generated 67 g L-lactate l^{-1} from xylose in 72 h (Tamakawa et al. 2012). The yeast *Pichia stipitis* is advantageous because it naturally ferments xylose, primarily to ethanol (Prior et al. 1989). Using *P. stipitis* with an L-LDH gene from *Lb. helveticus* expressed under the control of the ADH1 promoter led to 58 g L-lactate l^{-1} from 100 g xylose l^{-1} or 41 g lactate l^{-1} from 94 g glucose l^{-1} (Ilmén et al. 2007). The lower production of lactate from glucose was attributed to the greater ethanol formation from this substrate. Despite the presence of an intact pyruvate-to-ethanol pathway, the cells generated 70 % less ethanol from xylose with *ldhL* expression. Interestingly, this *P. stipitis* construct was able to metabolize glucose and xylose simultaneously.

Decrease of by-products from glycerol

Glycerol has also been used as a substrate for lactic acid production by *E. coli* (Mazumdar et al. 2010). By overexpressing enzymes involved in the conversion of glycerol to dihydroxyacetone phosphate, and knocking out fumarate reductase (*frdA*), phosphotransferase (*pta*), alcohol dehydrogenase (*adhE*) and respiratory D-lactate dehydrogenase (*dld*) genes, 32 g D-lactate l^{-1} was generated from 40 g glycerol l^{-1} in about 72 h in shake-flasks. Modifying the *E. coli* strain by replacing the native D-LDH with an L-LDH, blocking the methylglyoxal pathway, and preventing reassimilation of L-lactate by eliminating a native aerobic L-LDH led to 50 g L-lactate l^{-1} with 99.9 % optical purity from 56 g crude glycerol l^{-1} in 84 h (Mazumdar et al. 2013). An economic assessment of the conversion of glycerol to D-lactic acid by engineered *E. coli* indicated that the use of crude glycerol represents less than 8 % of the production cost, and that the process could be a profitable use for glycerol (Posada et al. 2012). Moreover, the success of the conversion depends mostly on the fermentation, and in particular the ability of strains to convert completely high concentrations of glycerol. Another approach involves the typical two-phase process: using a two-phase approach without overexpression of genes involved in glycerol uptake, *E. coli* was able to generate 99 g D-lactate l^{-1} in 36 h at a yield of 0.64 g/g (Tian et al. 2012; Chen et al. 2014). Overexpression of *ldhA* increased final concentration and yield. Importantly, a two-phase approach allows different environmental conditions: for example, *E. coli* growth is favored at 34–37 °C, while anaerobic conversion of glycerol into lactic acid favors 40 °C (Chen et al. 2014). The use of crude glycerol compared to purified glycerol does not appear to impact the production of lactic acid (Chen et al. 2014), although methanol present as high as 28 % (w/w) is largely lost on autoclaving (Pyle et al. 2008).

Wild-type *Klebsiella pneumoniae* generates a surprisingly high concentration of D-lactic acid when subject to microaerobic conditions and high glycerol feed rates (Rossi et al. 2013). If one thus accepts the generation of by-products, the organism is suitable for the co-production of 1,3-propanediol and D-lactic acid,

which may readily be separated from each other (Song et al. 2013). Knocking out just two genes leading to 1,3-propanediol (*dhaT* and *yqhD*) and overexpressing a D-LDH led to 142 g D-lactic acid l^{-1} at a yield of 0.82 g/g glycerol (Feng et al. 2014). The availability of some oxygen under microaerobic conditions appears to be critical in maximizing D-lactic acid formation in *K. pneumoniae* (Feng et al. 2014).

Membrane transport associated with lactate formation

In many cells glucose is transported across the cell membrane via a sugar-specific phosphotransferase system (PTS), though variations in this strategy exist. *Lc. lactis* transports glucose via a mannose-PTS, a cellobiose-PTS (rather than a glucose-PTS) and a unique glucose permease (Castro et al. 2009). The concentration of phosphate can play an important role in the regulation of glycolysis (e.g., *Lc. lactis*), with low phosphate inhibiting glycolysis particularly at high glucose concentrations (Levering et al. 2012). Xylose transport in several *Lactobacillus* spp. occurs via facilitated diffusion (Chaillou et al. 1998, 1999), while in *E. coli* xylose uptake occurs by both high-affinity (ATP-dependent) and low-affinity (ATP-independent) routes (Sumiya et al. 1995).

Saccharomyces cerevisiae appears to be limited in the efflux of lactate, and two monocarboxylate permeases, Jen1 and Ady2, play an important role in lactate export from the cell (Pacheco et al. 2012), though Ady2 is considered an acetate permease (Paiva et al. 2004). A knockout in both *JEN1* and *ADY2* significantly reduced consumption of lactic acid from the medium after the glucose had been exhausted while the overexpression of *JEN1* and *ADY2* increased lactic acid production but also increased lactic acid utilization after glucose exhaustion. Thus, these enzymes are involved in bidirectional lactic acid uptake and efflux (Pacheco et al. 2012). A mutation in the *ADY2* gene has also been linked to increased lactate efflux through laboratory evolution (de Kok et al. 2012). Although the quantity of lactic acid generated varies widely depending on the strain of yeast used (Branduardi et al. 2006), these earlier studies generated less than 0.5 g lactic acid/l (Pacheco et al. 2012). An almost doubling of lactate yield to 0.75 g/g glucose under continuous culture conditions led to speculation that this operational mode may be advantageous for lactic acid production by *S. cerevisiae* because such a process maintains a very low glucose concentration and thus reduces the Crabtree effect found in this yeast (Mimitsuda et al. 2014).

Compared to research involving upper or lower metabolism, relatively little research has investigated improving lactate production through the alteration of native sugar or lactate transport into or out of the cell.

Increasing microbial tolerance

Microbial processes for lactic acid generation are commonly limited by the tolerance of production organisms to extreme conditions of pH or temperature, as well to elevated sugar and lactic acid concentrations. Furthermore, inhibitors present in the biomass hydrolysates and other inexpensive materials which are attractive for lactic acid production can hinder growth and product formation. Therefore, the selection and improvement of strains for tolerance to certain environmental conditions are key aspects in process development for large scale cultivation. Two general strategies have been adopted for obtaining more tolerant microbial strains. One method involves improving known lactic acid producers by various evolutionary techniques such as random mutagenesis or genome shuffling. A second method is to establish first what characteristics are needed in a production microbe, and then isolate microbes from the natural environment which satisfy those characteristics. Lactic acid production is then subsequently introduced or improved in those isolates.

Tolerance to low pH

During microbial generation of lactic acid from simple sugars such as glucose, the pH of the culture will decrease. Microbes are often inhibited by lowered pH, leading to a reduction and ultimately a cessation of cell growth and further lactic acid formation. This pH-dependent inhibition necessitates the use of strong base to neutralize the accumulating acid during the course of the fermentation. However, the use of bases such as NaOH, KOH or $Ca(OH)_2$ is accompanied by its own drawbacks. In particular, the cells may be inhibited by the increased concentration of a neutralizing counter-cation (e.g., Na^+), and additional

processing steps are required to remove the counterion and leave the ultimate acid product. So, two approaches have been explored to resolve the problem of decreasing pH during a lactic acid fermentation, including increasing microbial tolerance to cations and increasing microbial tolerance to low pH.

Several researchers have increased tolerance to cations in microbes able to generate more lactate. Applying the hypothesis that Na^+ might limit lactic acid production, a 25 % increase in lactate accumulation (70 vs. 56 g l^{-1}) was reported in an *E. coli* strain overexpressing Na^+-specific regulator gene *nhaR* and the *nhaA* gene expressing the Na^+/H^+ antiporter membrane protein (Wu et al. 2013a). Similarly, overexpressing the DNA repair protein RecO in *L. casei* led to a 40 % increase in lactic acid concentration under NaCl stress, although the lactic acid concentrations accumulated were less than 5 g l^{-1} (Wu et al. 2013b). Subjecting a *Bacillus* isolate to low energy ions generated a mutant more tolerant to Na^+ which at 50 °C was able to convert glucose to over 100 g L-lactate l^{-1} with productivities exceeding 3.5 g l^{-1} h^{-1} (Qin et al. 2010). *E. coli* merely exposed to progressively increasing concentrations of Na^+ showed greater tolerance to both Na^+ and K^+ ions and, when conferred with a single knockout in the *pflB* gene, showed a 35 % increase in lactic acid production (76 g l^{-1}) compared to the wild-type strain (Wu et al. 2014).

Lactic acid-producing bacteria may similarly be adapted to greater acid tolerance. Just one round of whole genome shuffling on *Lb. pentosus* was necessary to generate transformants with improved growth at a pH of 4 (Ye et al. 2013a). One mutant, MT3, produced lactic acid at 95 % yield from 20 g glucose l^{-1} at a pH of 3.8. These authors did not identify the altered genes nor the mechanism for the increased acid tolerance but the mutation did not have any effect on lactate synthesis. Increased acid tolerance of *Lb. rhamnosus* was also achieved by genome shuffling using two UV mutants and three nitrosoguanidine mutants as the template strains (Wang et al. 2007). At a controlled pH of 4.5, the ultimate strain was able to generate about 84 g l^{-1} after an 84 h fermentation. Similarly, *Sporolactobacillus inulinus* was subject to UV irradiation and diethyl sulfate mutagenesis to generate starting populations for genome shuffling, which led to a strain resistant to a pH of 5 that generated 93 g D-lactic acid l^{-1} from glucose in complex medium (Zheng et al. 2010). Interestingly, a pH-tolerant mutant of *Lb. casei* showed greater PTS activity, greater H^+-ATPase activity and, at lower values of pH, maintained a higher intracellular pH than the wild-type, with a subsequent proteomic analysis demonstrating the complexity of pH tolerance (Wu et al. 2012). The response of LAB to low pH involves surprisingly numerous genes or proteins (Rallu et al. 2000; De Angelis et al. 2001).

A source to isolate new acid-tolerant bacteria is the corn-processing facilities themselves. Acid-tolerant bacteria which produce lactic acid at a pH of 4.2 or less have been isolated directly from industrial wet mills (Carlson and Peters 2002), and then subsequently examined for the effect of temperature, SO_2, and steep-water on their growth. Similarly, bacteria were isolated from corn-steep liquor producing L-lactic acid (Eddington et al. 2007), and six *Lactobacilli* were identified. These strains each generated over 80 g L-lactic acid l^{-1} with 96 % optical purity and a yield over 80 % in 50 h. After chemical mutagenesis, the strains were examined for the absence of D-lactic acid. In this way, strains able to generate exclusively L-lactic acid at high yield and final concentration were also generated.

Tolerance to elevated temperatures

Increased tolerance to elevated temperature is also desirable for potentially increasing productivity and for reducing the prospect of contamination. To increase thermotolerance of *Lb. casei*, a mutant was selected by isolating for growth at progressively higher temperatures and glucose concentrations (Ge et al. 2011). The resulting mutant, named G-03, generated lactate maximally at 41 °C, whereas the parent strain generated maximal lactate at 34 °C. G-03 generated over 198 g lactate l^{-1} at 41 °C, approximately twice the concentration and productivity of the parent strain.

Thermotolerant strains have been isolated directly from the environment. For example, Qin et al. (2009) selected *Bacillus* 2-6 from 730 soil isolates that grew at 55 °C, and Bischoff et al. (2010) isolated 40 strains on a xylose-containing complex medium at 50 °C, and 16S rDNA showed all were *B. coagulans*. Demonstrating the advantage that high temperature provides against contamination, *Bacillus* 2–6 generated 182 g L-lactic acid l^{-1} in a medium of 20 g yeast

extract l^{-1} in a 30 l fed-batch process without sterilization (Qin et al. 2009) into which glucose was fed continuously. *Bacillus* 2–6 also generated 60–100 g L-lactic acid l^{-1} with over 99 % optical purity in repeated batch fermentations at an average of 0.9 g/g yield and productivities exceeding 1 g l^{-1} h^{-1}, though production was very dependent on yeast extract concentration (Zhao et al. 2010). During batch fermentation of 120 g xylose l^{-1}, thermotolerant isolate *B. coagulans* NL01 generated 80 g L-lactate l^{-1} in 72 h (Ouyang et al. 2012), while *B. coagulans* C106 generated 216 g from xylose l^{-1} during a fed-batch process (Ye et al. 2013b). Otto (2012) describe thermophilic strains (56 °C) of *B. coagulans* and *Geobacter* also tolerant to a pH of 5.6. Immobilization of a thermotolerant *Bacillus* extends the productivity of the process: approx. 70 g lactic acid l^{-1} from 80 g/l glucose during each of fifteen repeated fed-batch fermentations (Rosenberg et al. 2005). All these recent studies using *Bacillus* strains confirm research from a decade earlier demostrating lactic acid production by thermotolerant *Bacillus* spp. (Heriban et al. 1993; Ohara and Yahata 1996; Payot et al. 1999).

A few species other than *Bacillus* have been isolated from the environment for their thermotolerance. For example, an *L. delbrueckii* strain isolated from a sink tolerated 50 °C. Using continuous culture with membrane recycle at 43 °C, this strain generated 20 g D-lactic acid l^{-1} continuously from 20 g glucose l^{-1} at a productivity of 18 g l^{-1} h^{-1} and a yield of 1 g/g (Tashiro et al. 2011). In batch culture 87.4 g D-lactic acid l^{-1} accumulated in 168 h from 100 g glucose l^{-1}. However, *B. coagulans* is advantageous compared to *Lb. delbrueckii* in terms of higher operational temperature, productivity and simplicity of medium requirements (Michelson et al. 2006).

Tolerance to elevated sugar concentration

Increased tolerance to high sugar concentrations or increased generation of lactic acid are worthy targets for random mutation. *Lb. lactis* was mutated with UV and selecting for increased acid production by strains having a larger acid zone on plate (Joshi et al. 2010). Two additional rounds of UV irradiation led to strain RM2-24, which generated 120 g lactic acid l^{-1} from 200 g hydrolyzed cane sugar l^{-1} in 120 h. Genome shuffling to increase the glucose tolerance of *Lb. rhamnosus* resulted in a strain which generated 180 g L-lactic acid l^{-1} from 200 g glucose l^{-1} in 90 h (Yu et al. 2008).

Tolerance to multiple stresses

Strategies have also been used to obtain strains with increased tolerance to multiple stresses. For example, Patel et al. (2006) isolated many microbes from a wide range of sources, and selected strains which could ferment sugars in sugar cane bagasse hydrolysate at a pH of 5 and 50 °C, conditions which match conditions necessary for cellulase activity and would reduce the need for sterilization and require less cooling. Three strains of *B. coagulans* generating lactic acid as the major anaerobic product were isolated, and strain 36D1 has been the subject of continuing research. *B. coagulans* 36D1 generated over 150 g lactic acid l^{-1} from either 200 g glucose l^{-1} or 200 g xylose l^{-1} in about 200 h (Ou et al. 2011). Similarly, Rallu et al. (2000) obtained 21 insertional mutants of *Lc. lactis* from a combination of low pH, elevated temperature and aerobic conditions. The researchers classified these mutants into four classes and concluded that acid tolerance is a complex process which involves phosphate and purine nucleotide pools and multiple transporters.

Heterologous expression of nisin-induced *dnaK* from *E. coli* in *Lc. lactis* improves tolerance to heat, salt and ethanol stress, particularly at 40 °C (Al-Mahin et al. 2010), though the concentrations of lactic acid were low (1–2 g l^{-1}). DnaK is believed to transduce signals to other cellular factors in response to increased temperature, and also to plays a role in refolding of thermally damaged proteins.

Further research on tolerant strains

Research has progressed to decrease by-product formation in some tolerant strains, or to use these strains directly for the conversion of lignocellulosic hydrolysates. For example, thermotolerant *B. coagulans* P4-102B with knockouts in the native L-LDH (*ldh*) and acetolactate synthase (*alsS*) genes, regains anaerobic growth after a mutation in glycerol dehydrogenase (*gldA*) causes the enzyme to act as a D-LDH, resulting in 90 g D-lactate l^{-1} from glucose after 48 h (Wang et al. 2011). Interestingly, for *B. coagulans* NL01 both acetate and levulinate exhibited a

toxic effect, while 2 g formate l^{-1} showed a stimulatory effect, and in corn stover hydrolysate this strain consumed xylose, arabinose and glucose simultaneously (Ouyang et al. 2012). *B. coagulans* MXL-9 grew on a synthetic mixture of 17 g l^{-1} each of glucose, xylose and arabinose, resulting in about 90 % yield on the three sugars after 36 h in the pH range of 6–6.5 (Bischoff et al. 2010). MXL-9 showed a 50 % decrease in OD after 24 h with 2.5–5 g furfural l^{-1} and 5-HMF, but was nevertheless able to ferment corn fiber hydrolysates without overliming to over 40 g lactic acid l^{-1}. *Bacillus* XZL9, which was isolated specifically for L-lactic acid formation from xylose at 50 °C, generated 134 g L-lactic acid l^{-1} in 218 h in a fed-batch fermentation, and about 74 g L-lactic acid l^{-1} from corncob molasses (Wang et al. 2010b). Lactate-generating fungi have also been the subject of strain improvement: subjecting *R. oryzae* to low energy ions led to mutants with increased metabolism of both xylose and glucose to 80 g lactic acid l^{-1} in 70 h, although the rate of xylose conversion remained much slower than the rate of glucose conversion (Wang et al. 2009).

Conclusions

The microbial production of lactic acid is a maturing technology which has, for over a decade, been successfully commercialized by several companies. With a current production cost on the order of a dollar per kg (Posada et al. 2012), even the highest producing strains generate a culture having lactic acid valued at less than $0.25 per liter. At such a low value for the fermentation product, the cost of the medium and operating conditions become critical in achieving an economic target for lactic acid production. Significant improvements have been achieved by isolating new microbes, or enhancing existing microbes for greater tolerance to high temperature and low pH in order to reduce contamination and operational costs. Nevertheless, the practical goal of improving microbe characteristics should be accompanied by a fundamental understanding of how cells response to such environments (e.g., membrane integrity, lactate transport, enzyme stability). Bioinformatic tools, for example, though rarely applied for lactic acid production, could provide better enzymes and processes. With the consistent desirability for using inexpensive agriculturally-generated residues, and reducing the cost of the medium, research should continue to provide scalable understanding into cellular responses to such substrates. Research on bioreactor operational modes, such as nutrient-limited (N, P, etc.) fed-batch processes, could not only provide further insights into cellular responses to environmental conditions, but could make significant improvements in lactic acid productivity, yield and final concentration.

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