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REVIEW

Fermentative production of lactic acid from renewable materials: Recent achievements, prospects, and limits

Ying Wang,[1] Yukihiro Tashiro,[2,3] and Kenji Sonomoto[1,4,*]

Laboratory of Microbial Technology, Division of Applied Molecular Microbiology and Biomass Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan,[1] Institute of Advanced Study, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan,[2] Laboratory of Soil Microbiology, Division of Applied Molecular Microbiology and Biomass Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan,[3] and Laboratory of Functional Food Design, Department of Functional Metabolic Design, Bio-Architecture Centre, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan[4]

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The development and implementation of renewable materials for the production of versatile chemical resources have gained considerable attention recently, as this offers an alternative to the environmental problems caused by the petroleum industry and the limited supply of fossil resources. Therefore, the concept of utilizing biomass or wastes from agricultural and industrial residues to produce useful chemical products has been widely accepted. Lactic acid plays an important role due to its versatile application in the food, medical, and cosmetics industries and as a potential raw material for the manufacture of biodegradable plastics. Currently, the fermentative production of optically pure lactic acid has increased because of the prospects of environmental friendliness and cost-effectiveness. In order to produce lactic acid with high yield and optical purity, many studies focus on wild microorganisms and metabolically engineered strains. This article reviews the most recent advances in the biotechnological production of lactic acid mainly by lactic acid bacteria, and discusses the feasibility and potential of various processes.

[**Key words:** Lactic acid; Renewable materials; Microbial fermentation; Lactic acid bacteria; Metabolic pathways]

Lactic acid (2-hydroxypropanoic acid) is a naturally occurring hydroxycarboxylic acid, first refined from sour milk by the Swedish chemist Scheele in 1780 (1). Subsequently, due to its versatile applications as an acidulant, flavour enhancer, and preservative, lactic acid has occupied a prime position in the food, pharmaceutical, cosmetic and other chemical industries (2,3). Recently, new uses for this compound are emerging. Lactic acid production has received a significant amount of interest because it can be used as a feedstock for the production of poly-lactic acid (PLA), a polymer present in medical applications and environmentally friendly biodegradable plastics, which can be substituted for synthetic plastics derived from petroleum resources (2–4). In nature, lactic acid occurs in two optical isomers, D (−)- and L (+)-lactic acids. L (+)-Lactic acid is the preferred isomer in the food and drug industries, because only this form is adapted to be assimilated by the human body. Therefore, the forms of lactic acid in pure isomers are more valuable for different specific applications (4,5). Copolymerization of the D (−)- and L (+)-isomers results in amorphous materials, whereas homopolymers form regular structures and are in a crystalline phase (6).

Lactic acid can be manufactured commercially by either chemical synthesis or biotechnological production by lactic acid fermentation. The most common method to synthesize lactic acid is based on the hydrolysis of lactonitrile. However, chemical synthesis always yields a racemic mixture of DL-lactic acid from petroleum resources. On the other hand, an optically pure L (+)- or D (−)-lactic acid can be obtained by the microbial fermentative method. Currently, approximate 90% of lactic acid is produced by the microbial fermentation. With the development of industrial bioconversion, microbial fermentation by the appropriate microorganism has become the dominant method of lactic acid production due to environmental concerns, low production temperature, low energy requirements, and high purity (7).

In recent times, the consumption of lactic acid as a feedstock for the production of PLA has increased considerably. However, the amount of PLA production (450 million kilograms per year) is still much lower than the total amount of plastics production (200 billion kilograms per year) (8). PLA production is restricted by high production costs, although the annual industrial investment is several million dollars (9). It has been reported that the cost of raw materials for the fermentative production of lactic acid usually accounts for greater than 34% of the total manufacturing cost (10). Thus, the efficiency and economics of lactic acid fermentation is still a problem from many points of view, and the substrate plays a vital role in the improvement of such a process. There have been various attempts to produce lactic acid efficiently from economic resources, such as rice bran (11), paper sludge (12), and green microalga (13). Nowadays, renewable materials such as lignocellulose and starch from agricultural residues and forestry resources are generally

* Corresponding author at: Laboratory of Microbial Technology, Division of Applied Molecular Microbiology and Biomass Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Tel./fax: +81 (0)92 642 3019.
E-mail address: sonomoto@agr.kyushu-u.ac.jp (K. Sonomoto).

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considered to represent an attractive substrate as feedstock for the production of lactic acid due to their abundance (5). However, one bottleneck for lactic acid production utilizing renewable materials is the cost of pretreatment. Most renewable materials are not directly available for lactic acid fermentation without pretreatment due to their intimate association with lignin and the lack of hydrolytic enzyme production by lactic acid-producing strains (5). Another limiting factor is the recovery and purification of lactic acid from the fermentation broth, because complex media consisting of various nutrients hampers not only the separation but also the purification of lactic acid. Therefore, there are many challenges in the industrial bioconversion of renewable materials to lactic acid.

This article presents a review of recent advances in the biotechnological production of lactic acid using renewable materials from the aspects of metabolic and enzymatic mechanisms, and metabolic engineering associated with lactic acid production. The major production processes, renewable materials, bioreactor systems, and fermentation modes are reviewed. We also describe recent achievements and limitations in simultaneous saccharification and fermentation (SSF) and molecular genetic approaches in the production of lactic acid from renewable materials.

MICROORGANISMS INVOLVED IN BIOTECHNOLOGICAL PRODUCTION OF LACTIC ACID

Wild type of LAB Lactic acid bacteria (LAB) are defined as facultative anaerobic or micro-aerophilic organisms and characterized by the following aspects: (i) can grow at temperatures as low as 5°C or as high as 45°C, (ii) can grow at pH 4.0–4.5, some also proliferate at pH 3.2 or 9.6, and (iii) generally require complex nitrogens, vitamins, and minerals for growth and lactic acid production (14). Studies on LAB constitute approximately 90% of the literature on lactic acid production because they can produce lactic acid with high yield and high productivity. The most common LAB species belong to the genera *Lactobacillus*, *Lactococcus*, *Pediococcus*, *Aerococcus*, *Carnobacterium*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, *Weisella*, *Leuconostoc*, *Streptococcus*, and *Enterococcus*. Among them, optically pure L (+)-lactic acid is produced by several species such as *Enterococcus mundtii* (15–17) and *Lactococcus lactis* (18), while D (−)-lactic acid can be produced by *Lactobacillus delbrueckii* (19).

Wild-type LAB, isolated and screened from various sources, are always the most powerful source for obtaining fermentable and genetically stable strains, which are widely utilized in lactic acid production. Two *Lactobacillus* strains of OND 32T and YAM 1 were isolated from sour cassava starch fermentation, and can directly ferment starchy biomass to lactic acid (20). Abdel-Rahman et al. (16,17) presented a novel wild-type strain of *E. mundtii* QU 25, which is a very attractive candidate for efficiently metabolizing lignocellulose-derived sugars into optically pure homo L (+)-lactic acid. The strain can produce lactic acid from a high concentration of xylose via the pentose phosphate pathway (16) as described in detail in next section, through which 3 mol of xylose yield 5 mol of lactic acid. This means that the theoretical yield is close to 100%, and few by-products are formed through fermentation by this strain (16).

Wild type of *Bacillus* genus and fungi Although LAB are widely used in lactic acid production, some other strains such as those of the genus *Bacillus*, as well as fungi, also produce lactic acid. Patel et al. (21) isolated *Bacillus* sp. strains 17C5 and 36D1 from soil and geysers, and proved their ability to produce L (+)-lactic acid from sugarcane bagasse with a maximum productivity of 6.7 mmol L^{-1} h^{-1} in SSF. Another group focused on the bioconversion of paper sludge to lactic acid by the thermophilic *Bacillus coagulans* strain P4-102B (12).

The best-known fungal source as a lactic acid producer is *Rhizopus oryzae*. In general, *R. oryzae* has relatively lower nutritional demands but the mycelial morphology and oxygen supply are considered to influence lactic acid productivity. The first report on efficient D (−)-lactic acid fermentation by *R. oryzae* was in 1936 (22). *R. oryzae* NRRL 395 has been recognized as one of the most suitable fungi for lactic acid fermentation (23). Guo et al. (24) described a fermentation process involving the simultaneous utilization of hemicellulose and cellulose in corncobs by a newly isolated *R. oryzae*.

Engineered microorganisms by mutagenesis and metabolic engineering Studies also focus on engineered microorganisms in order to meet the commercial requirements including improved optical purity of the product, reduction of nutritional supply, improved yield and productivity, a broader substrate specificity, and the elimination of plasmids and antibiotic markers. The initial efforts of genetic modifications were mainly on improving LAB by traditional approaches, which involve exposing the bacterium to mutagens such as ethylmethyl sulphonate, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, and ultraviolet radiations (25,26).

On the other hand, efficient genetic tools targeting several microorganisms have been developed over the past few decades. Amongst these, metabolic engineering is an important tool for industrial biotechnology (27). According to the manipulation of enzyme functions, transcription, and the regulatory system in the microorganisms, metabolic engineering redirects metabolic flux, changes cellular protein levels, and regulates gene expression in several hosts such as *Saccharomyces cerevisiae* (28), *Escherichia coli* (29), *Corynebacterium glutamicum* (30), *R. oryzae* (31), and *Lactococcus lactis* (32). Table 1 shows a summary of recent studies on engineering approaches for lactic acid production.

STUDIES ON METABOLIC PATHWAYS WITH LAB

LAB ferment sugars such as hexose and pentose via different metabolic pathways that lead to homo- or hetero-fermentation (Fig. 1). Homo-fermentation produces virtually only lactic acid as the end product via the Embden–Meyerhof–Parnas (EMP) pathway and the pentose phosphate (PP)/glycolic pathway from hexose and pentose, respectively. The theoretical yield of lactic acid from glucose is 1.0 g g^{-1} (2.0 mol mol^{-1}) via the EMP pathway while pentose exhibits a theoretical yield of 1.0 g g^{-1} (1.67 mol mol^{-1}) of lactic acid via the PP/glycolic pathway (43,44). In the EMP pathway, the first steps of glycolysis are the phosphorylation of glucose to fructose 1,6-diphosphate (FDP) and its subsequent cleavage into dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). The GAP is then converted to pyruvate via a route that includes 2 substrate-level phosphorylation steps. Finally, pyruvate is reduced to lactic acid by L-LDH or D-LDH with the oxidation of NADH to NAD^+ for the redox balancing. In the PP/glycolic pathway, 3 mol of xylulose 5-phosphate (xylulose 5-P; 5 carbons), generated by the phosphorylation of pentose sugars such as xylose and arabinose, is converted to 5 mol of GAP (3 carbons) via 2 key enzymes: transketolase and transaldolase. The resulting GAP is converted to pyruvate and then to lactic acid (3 carbons) as the final product, thereby providing a theoretical yield of lactic acid from pentose of 1.0 g g^{-1} (1.67 mol mol^{-1}).

On the other hand, in hetero-fermentation, equimolar amounts of lactic acid, carbon dioxide, and ethanol (or acetate) are formed via the phosphoketolase (PK) pathway, in which glucose 6-phosphate (6 carbons) is initially converted to ribulose 5-phosphate (5 carbons) and carbon dioxide (1 carbon) in a reaction catalyzed by several enzymes (45). Then, the resulting xylulose 5-P from ribulose 5-phosphate is cleaved to an equimolar amount of GAP and acetyl phosphate (acetyl-P). The acetyl-P is reduced to ethanol