Novel D-lactic acid-producing strain and use thereof

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a novel D-lactic acid-producing strain and a use thereof. Specifically, the present invention relates to a method for preparing a D-lactic acid-producing strain including the steps of inhibiting L-lactate dehydrogenase (L-LDH) activity and introducing D-lactate dehydrogenase (D-LDH) activity in an L-lactic acid-producing strain, a modified D-lactic acid-producing strain prepared by the above method, and a method for producing D-lactic acid including the steps of culturing the strain and recovering D-lactic acid from the culture broth.

2. Description of the Related Art

Lactic acid has a wide range of industrial applications in foods, medicines, cosmetics, etc. In recent years, lactic acid has been utilized as a monomer of polylactic acid, and thus there has been a remarkable increase in demand for lactic acid.

Lactic acid can be produced by the chemical synthesis or the biological fermentation process using carbohydrates

as a substrate. The latter is preferred from a commercial point of view because the chemical synthesis of lactic acid creates a problem of the cost increase caused by the gas price increase or environmental contamination. In addition, there are also problems of producing L-lactic acid in the form of a racemic mixture consisting of an equal amount of D-lactic acid and L-lactic acid.Unfortunately, the composition ratio of the D-lactic acid and the L-lactic acid cannot be controlled. When lactic acid in the form of a racemic mixture is used for preparing polylactic acid, an amorphous polymer with a low melting point is produced, thus an application of it is limited. On the other hand, the biological fermentation process using microorganisms makes it possible to selectively produce D- or L-lactic acid depending on the strain used. For example, microorganisms such as *LactoBacillus sp.*, *Bacillus sp.*, *Rhizopus sp.*, *Streptococcus sp.*, or *Enterococcus sp.* usually produce L-lactic acid. Microorganisms such as *Leuconostoc sp.* and *Lactobacillus vulgaricus* usually produce D-lactic acid. In particular, due to D-lactic acid is not metabolized in the body, D- lactic acid can be used as a biomaterial in the medical field and also used as an optically active herbicide via esterification and chlorination. It has been known that an optically active herbicide, , can considerably improve its pharmaceutical effect and also has the same

pharmaceutical effect with a lesser amount. For this reason, a demand for D-lactic acid has been increasing. In addition, sc-polylactic acid (stereocomplex-PLA) has a significantly higher melting point and thermal degradation temperature than the known polylactic acids. Therefore, it can be used as a high heat-resistant plastic material, resulting from a mixture of pure L-polylactic acid and pure D-polylactic acid. Consequently, a monomer of D-lactic acid is needed, and its demand has been gradually growing.

In producing such optically pure D-lactic acid, the biological fermentation process using enantioselective substrate specificity of a microbial enzyme is preferred. However, the wild-type, D-lactic acid-producing microorganisms generally found in nature, are still not suitable for industrial use regarding of optical purity or productivity. Examples of the D-lactic acid-producing microorganisms are *Lb. plantarum*, *Lb. pentosus*, *Lb. fermentum*, *Lb delbrueckii*, or the like. However, there are disadvantages that they are not able to produce lactic acid with high productivity and high yield, and 20~40% of the lactic acid is L-lactic acid as an optical impurity. To overcome these disadvantages, attempts have been made to develop a variant producing high concentrations of lactic acid in a high glucose medium by inducing mutations in lactic acid-producing bacteria with treatment of EMS (ethyl

methanesulfonate) (*J. Industrial Microbiol*, 11:23-28, 1992). As a result, the strain showing about a 4.8-fold higher productivity than a control group was selected, but its activity was reduced during long-term storage. Meanwhile, in the case of strain development using a variant, a yield-improved strain tends to show reduced productivity, whereas a productivity-improved strain tends to show reduced yield.

Based on the idea that strains for industrial lactic acid fermentation are generally L-lactic acid-producing microorganisms, and these microorganisms have mostly superior productivity and yield compared to D-lactic acid-producing microorganisms , the present inventors found that D-lactic acid can be produced in high yield by inactivating an L-lactate dehydrogenase (L-LDH)-encoding gene in a high L-lactic acid-producing microorganism and then introducing a heterogenous D-lactate dehydrogenase (D-LDH)-encoding gene thereto, thereby completing the present invention.

SUMMARY OF THE INVENTION

An embodiment of the present invention provides a method for preparing a modifiedD-lactic acid-producing strain, including the steps of attenuating or inactivating L-lactate dehydrogenase activity and introducing or

enhancing D-lactate dehydrogenase activity in an L-lactic acid-producing strain.

Other embodiment of the present invention provides a modified D-lactic acid-producing strain that is prepared by the above method.

Another embodiment of the present invention provides a method for producing D-lactic acid using the modified D-lactic acid-producing strain.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a graph representing the results of analyzing a ratio of D-lactic acid and L-lactic acid produced by 10 different types of wild-type lactic acid-producing microorganisms.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

An embodiment of the present invention provides a method for preparing a modified D-lactic acid-producing strain by attenuating or inactivating L-lactate dehydrogenase (L-LDH) activity and introducing or enhancing D-lactate dehydrogenase (D-LDH) activity in an L-lactic acid-producing strain.

In detail, the method for preparing the modified D-lactic acid-producing strain of the present invention

includes (a) attenuating or inactivating L-lactate dehydrogenase (L-LDH) activity in an L-lactic acid-producing strain to obtain a modified lactic acid-producing strain; and (b) introducing or enhancing D-lactate dehydrogenase (D-LDH) activity in the modified lactic acid-producing strain.

In this regard, the L-lactic acid-producing strain may be a strain expressing only an L-LDH-encoding polynucleotide to produce L-lactic acid or a strain expressing an L-LDH-encoding polynucleotide and a D-LDH-encoding polynucleotide at the same time to produce both L-lactic acid and D-lactic acid. The method for attenuating or inactivating L-LDH activity may be carried out by substituting, deleting, inserting or adding one or several nucleotides at one or more positions of the L-LDH-encoding polynucleotide. The method for introducing or enhancing D-LDH activity may be carried out by introducing the D-LDH-encoding polynucleotide into the chromosome of the modified lactic acid-producing strain, introducing a polynucleotide encoding a D-LDH variant having improved activity into the chromosome of the modified lactic acid-producing strain, introducing a strong promoter into upstream of the D-LDH-encoding polynucleotide in the chromosome of the mutated lactic acid-producing strain, introducing a strong promoter and the D-LDH-encoding polynucleotide operably linked to the promoter into the chromosome of the modified lactic acid-producing strain,

introducing a strong promoter and the polynucleotide encoding the D-LDH variant having improved activity, which is operably linked to the promoter, into the chromosome of the modified lactic acid-producing strain, introducing an expression vector including the D-LDH-encoding polynucleotide into the modified lactic acid-producing strain, introducing an expression vector including the polynucleotide encoding the D-LDH variant having improved activity into the modified lactic acid-producing strain, introducing an expression vector including a strong promoter and the D-LDH-encoding polynucleotide operably linked to the promoter into the modified lactic acid-producing strain, introducing an expression vector including a strong promoter and the polynucleotide encoding the D-LDH variant having improved activity, which is operably linked to the promoter, into the modified lactic acid-producing strain, or the like.

As used herein, the term "lactate dehydrogenase (LDH)" refers to an enzyme that catalyzes production of pyruvate from lactate by removal of hydrogen or production of lactate from pyruvate by reductionusing NADH. LDH has a molecular weight of about 140 kDa, and can be classified into L-LDH (EC 1.1.1.27.) producing L-lactic acid, D-LDH (EC 1.1.1.28.) producing D-lactic acid, and L-LDH (cytochrome b2, EC 1.1.2.3) containing FMN and heme.

As used herein, the term "L-lactic acid-producing strain" refers to a strain that expresses an L-LDH-encoding polynucleotide and produces L-lactic acid using the expressed L-LDH. In addition, the strain also includes a strain that produce both L-lactic acid and D-lactic acid by expressing the L-LDH-encoding polynucleotide and D-LDH-encoding polynucleotide at the same timeas a strain that produce L-lactic acid by expressing only the L-LDH-encoding polynucleotide. The L-lactic acid-producing strain is not particularly limited, as long as it can produce L-lactic acid. For example, *Lactobacillus brevis*, *Lactobacillus pentosus*, *Lactobacillus rhamnosus*, *Lactobacillus jensenii*, *Lactobacillus plantarum*, *Lactobacillus paraplantarum*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, and *Lactobacillus casei* may be used, specifically, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, and *Lactobacillus casei* may be used, and more specifically *Lactobacillus paracasei* may be used.

The method for attenuating or inactivating L-LDH activity in the L-lactic acid-producing strain may be carried out using a method known in the art. For example, a method for inhibiting expression of the L-LDH-encoding

polynucleotide or producing inactivated L-LDH may be a method for substituting, deleting, inserting or adding one or several nucleotides, specifically 2 to 20 nucleotides, more specifically, 2 to 10 nucleotides, and further more specifically 2 to 5 nucleotides of the L-LDH-encoding polynucleotide inherent in the L-lactic acid-producing strain. In addition, any method may be used without particular limitation, as long as it is used to attenuate or inactivate L-LDH activity in the L-lactic acid-producing strain.

In the present invention, L-LDH to be attenuated or inactivated may inherent in the L-lactic acid-producing strain. The amino acid sequence of the L-LDH or the polynucleotide sequence encoding the same is not particularly limited. The L-LDH may be represented by a polynucleotide sequence (SEQ ID NO: 25) encoding LDH and a polynucleotide sequence (SEQ ID NO: 26) encoding LDH1 of *Lactobacillus paracasei*, a polynucleotide sequence (SEQ ID NO: 27) encoding LDH1 and a polynucleotide sequence (SEQ ID NO: 28) encoding LDH2 of *Lactobacillus casei*, a polynucleotide sequence (SEQ ID NO: 29) encoding LGG_02523 and a polynucleotide sequence (SEQ ID NO: 30) encoding LGG_00606 of *Lactobacillus rhamnosus*.

As used herein, the term "modified lactic acid-producing strain" refers to an L-lactic acid-producing strain of which L-LDH activity is attenuated or inactivated. The lactic acid-producing strain may be modified to attenuate or inactivate L-LDH activity by inducing artificial mutations in the normal L-lactic acid-producing strain, or by naturally occurring mutations without inducing artificial mutations.

The method for introducing or enhancing D-LDH activity in the modified lactic acid-producing strain may be, but is not particularly limited to, a method for introducing the D-LDH-encoding polynucleotide into the chromosome of the modified lactic acid-producing strain, a method for introducing a polynucleotide encoding a D-LDH variant having improved activity into the chromosome of the mutated lactic acid-producing strain, a method for introducing a strong promoter into upstream of the D-LDH-encoding polynucleotide in the chromosome of the modified lactic acid-producing strain, a method for introducing a strong promoter and the D-LDH-encoding polynucleotide operably linked to the promoter into the chromosome of the modified lactic acid-producing strain, a method for introducing a strong promoter and the polynucleotide encoding the D-LDH variant having improved activity, which is operably linked to the promoter,

into the chromosome of the modified lactic acid-producing strain, a method for introducing an expression vector including the D-LDH-encoding polynucleotide into the modified lactic acid-producing strain, a method for introducing an expression vector including the polynucleotide encoding the D-LDH variant having improved activity into the modified lactic acid-producing strain, a method for introducing an expression vector including a strong promoter and the D-LDH-encoding polynucleotide operably linked to the promoter into the modified lactic acid-producing strain, a method for introducing an expression vector including a strong promoter and the polynucleotide encoding the D-LDH variant having improved activity, which is operably linked to the promoter, into the modified lactic acid-producing strain, or the like.

As used herein, the term "expression vector" refers to a DNA product comprising a nucleotide sequence of a polynucleotide encoding a target protein, which is operably linked to a suitable regulatory sequence to express the polynucleotide encoding the target protein in a suitable host. The regulatory sequence may include a promoter capable of initiating transcription, an arbitrary operator sequence for regulating transcription, a sequence encoding an appropriate mRNA ribosome binding site, and sequences for

regulating the termination of transcription and translation. Once a vector is transformed into a suitable host, the vector may replicate and function independently of the host genome, or may be integrated into the genome itself.

As long as it is replicable in hosts, any vector known in the art may be used as the expression vector in the present invention, without particular limitations. Example of the expression vector typically used may include a natural or recombinant plasmid, cosmid, virus and bacteriophage. Example of the phage vector or the cosmid vector may include *pWE15, M13, λMBL3, λMBL4, λIXII, λASHII, λAPII, λt10, λt11, Charon4A,* and *Charon21A*. The plasmid vector may include *pBR* type, *pUC* type, *pBluescriptII* type, *pGEM* type, *pTZ* type, *pCL* type, *pET* type, etc.

In detail, the vector used in embodiments of the present invention is pG+host6 which is a vector used in a wide range of Gram-positive bacteria. This vector is characterized in that it contains an ampicillin-resistant gene and a replication origin for use in *E. coli*, an erythromycin-resistant gene and a replication origin for use in Gram-positive bacteria. In particular, the replication origin in Gram-positive bacteria contains a heat-sensitive mutation, and therefore, replication does not occur at a

temperature above 37°C. Therefore, itallows gene insertion via a homologous sequence in Gram-positive bacteria (US Patent Application Publication No. 20060025190).

As used herein, the term "transformation" means a series of operations of introducing a vector including a polynucleotide encoding a target protein into a host cell, expressing the polynucleotide in the host cell, and producing an expression product, mRNA or protein. The polynucleotide to be introduced into the host cell may be in any form, as long as it can be introduced into the host cell and expressed therein. For example, the polynucleotide may be introduced into a host cell in the form of an expression cassette that is a structure including all elements (a promoter operably linked to the polynucleotide, a transcription termination signal, a ribosome binding site, a translation termination signal, etc.) required for self-expression. The expression cassette may be in the form of a self-replicable expression vector. In addition, the polynucleotide itself may be introduced into a host cell to be operably linked to a sequence required for expression in the host cell.

D-LDH used in the present invention may be, but is not particularly limited to, derived from a strain producing D-

lactic acid. Specifically derived from *Lactobacillus plantarum* or *Lactobacillus delbrueckii*, and further more specifically, a polypeptide represented by an amino acid sequence of SEQ ID NO: 31 of *Lactobacillus delbrueckii* or an amino acid sequence of SEQ ID NO: 32 of *Lactobacillus plantarum*. In addition, substitution, deletion, insertion, addition or inversion of one amino acid or several amino acids (may be depending on positions of amino acid residues in the three-dimensional structure of the protein or types of the amino acid residues, specifically 2 to 20, more specifically 2 to 10, further more specifically 2 to 5 amino acids) at one or more positions of amino acid sequence of SEQ ID NO: 31 or 32, may be included. As long as it can maintain or enhance the D-LDH activity, an amino acid sequence having 80% or more, specifically 90% or more, more specifically 95% or more, further more specifically 97% or more homology with the amino acid sequence of SEQ ID NO: 31 or 32 may be included. Since the amino acid sequence of the enzyme may be different depending on the species or the strain of a microorganism, the substitution, deletion, insertion, addition or inversion of the amino acid also includes a naturally occurring mutated sequence or an artificially mutated sequence, but is not particularly limited thereto.

As used herein, the term "homology" refers to identity between two different amino acid sequences or two different nucleotide sequences, and can be determined by a method well known to those skilled in the art. For example, BLAST 2.0calculating parameters such as score, identity, and similarity may be used, but is not particullimited thereto.

Generally, the L-lactic acid-producing strain produces lactic acid with a higer production yield than the D-lactic acid-producing strain. Thus, the present inventors intended to prepare a strain having excellent D-lactic acid producibililty by modifying the L-lactic acid-producing strain to the D-lactic acid-producing strain. To this end, the fermentation ratio of D- and L-lactic acids was compared between the wild-type *LactoBacillus sp.* strains. As a result, it was found that *Lactobacillus paracasei*, *Lactobacillus casei* and *Lactobacillus rhamnosus* strains showed excellent overall producibility of lactic acid, and their L-lactic acid ratios were overwhelmingly excellent. Therefore, the present inventors intended to prepare modified strains thereof (FIG. 1). For example, *ldh* and *ldh1* genes of L-LDHin *Lactobacillus paracasei* were deleted, and at the same time, δldh1-ldhA(*Lb. db*) and δldh-ldhD(*Lb. pl*) as the cassettes for D-LDH insertion were prepared, and then each of them was introduced into the heat-sensitive

vector pG+host6 to prepare two types of vectors, *pG+host6-δldh1-ldhA*(*Lb. db*) and *pG+host6-δldh-ldhD*(*Lb. pl*) (Example 3). Subsequently, each vector was introduced into L-LDH gene-deleted *Lactobacillus paracasei* to prepare a transformant modified to attenuate or inactivate L-LDH activity and to enhance D-LDH activity (Example 4). Thus the prepared transformants were cultured, and lactic acid produced therefrom was analyzed. As a result, D-lactic acid was produced at a concentration of 41.6 g/L, but no L-lactic acid was produced. The production yield of D-lactic acid produced in the present invention was higher than that of D-lactic acid produced by the known D-lactic acid-producing strain (Example 5).

Accordingly, when L-LDH activity is attenuated or inactivated and D-LDH activity is introduced or enhanced in the L-lactic acid-producing strain having high production yield of lactic acid, D-lactic acid can be produced in higher yield than c the known D- lactic acid-producing strains.

An embodiment of the present invention provides a D-lactic acid-producing strain modified to attenuate or inactivate the L-LDH activity and to introduce or enhance the D-LDH activity in the L-lactic acid-producing strain showing the L-LDH activity using the above method.

The modified D-lactic acid-producing strain may be a strain in which the D-LDH-encoding polynucleotide is substituted for the L-LDH-encoding polynucleotide in the chromosome of L-lactic acid-producing strain or is overexpressed. The modified D-lactic acid-producing strain, although not particularly limited, may be a strain in which a polynucleotide encoding LDH1 (SEQ ID NO: 27) and a polynucleotide encoding LDH2 (SEQ ID NO: 28) of *Lactobacillus casei* are substituted with a polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and a polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*, respectively; a strain in which a polynucleotide encoding LDH(LGG_02523) (SEQ ID NO: 29) and a polynucleotide encoding LDH(LGG_00606) (SEQ ID NO: 30) of *Lactobacillus rhamnosus* are substituted with a polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and a polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*, respectively; a strain in which a polynucleotide encoding LDH (SEQ ID NO: 25) and a polynucleotide encoding LDH1 (SEQ ID NO: 26) of *Lactobacillus paracasei* are substituted with a polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and a polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*, respectively.

Specifically, The modified D-lactic acid-producing strain may be a strain in which a polynucleotide encoding LDH (SEQ ID NO: 25) and a polynucleotide encoding LDH1 (SEQ ID NO: 26) of *Lactobacillus paracasei* are substituted with a polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and a polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*, respectively, and more specifically, *Lactobacillus paracasei* CC02-0095(KCCM11273P).

The present inventors produced D-lactic acid using each of the transformants which were modified to delete each of the L-LDH-encoding polynucleotides in *Lactobacillus paracasei*, *Lactobacillus casei* and *Lactobacillus rhamnosus* strains and to introduce the polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and the polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*, respectively into them. The yield, productivity, production amount, etc., of the D-lactic acid were compared. As a result, it was confirmed that the transformant derived from *Lactobacillus paracasei* (*Lb. paracasei* ldh::ldhA ldh1::ldhD) was the most excellent in terms of yield, productivity, and production amount of the D-lactic acid.

Accordingly, the present inventors designated the transformant (*Lb. paracasei ldh::ldhA ldh1::ldhD*) as *Lactobacillus paracasei* CC02-0095, in which the transformant was the most excellent in terms of yield, productivity, and production amount of the D-lactic acid. The CC02-095was a strain prepared by substituting the polynucleotide encoding LDH (SEQ ID NO: 25) and the polynucleotide encoding LDH1 (SEQ ID NO: 26) of *Lactobacillus paracasei* with the polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and the polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*. The transformant was deposited with the Korean Culture Center of Microorganisms (hereinafter, abbreviated to "KCCM") under the Budapest Treaty on April 2, 2012 under Accession No. KCCM11273P.

Another embodiment of the present invention provides a method for producing D-lactic acid, including the steps of (a) culturing the modified D-lactic acid-producing strain to obtain a culture broth; and (b) recovering D-lactic acid from the culture broth.

The modified D-lactic acid-producing strain of the present invention is a strain prepared from an L-lactic acid-producing strain having excellent lactic acid

producibility in order to make the strain produce D-lactic acid. Therefore, when the modified D-lactic acid-producing strain is cultured, D-lactic acid produced may be accumulated within the strain or in the culture medium. Consequently, D-lactic acid may be obtained by recovering the D-lactic acid that is accumulated within the cultured strain or in the culture medium.

As used herein, the term "culture" means all of the actions to grow a microorganism under moderately controlled artificial environmental conditions. In the present invention, the culture is conducted for the purpose of producing D-lactic acid from the modified D-lactic acid-producing strain, and a specific method for the culture is not particularly limited, as long as it can produce D-lactic acid from the modified D-lactic acid-producing strain. It can be conducted using any method widely known in the art. Specifically, it can be conducted by a batch process, a fed batch process or a continuous process.

Specifically, the medium used for the culture may have to meet the requirements of a specific strain in a proper manner while controlling temperature, pH, etc. under aerobic conditions in a typical medium containing a proper carbon source, nitrogen source, amino acids, vitamins, etc.

Possible carbon sources may include a mixture of glucose and xylose as a main carbon source, sugars and carbohydrates such as sucrose, lactose, fructose, maltose, starch, and cellulose, , oils and fats such as soy bean oil, sunflower oil, castor oil, and coconut fat, fatty acids such as palmitic acid, stearic acid, and linoleic acid, alcohols such as glycerol and ethanol, and organic acids such as acetic acid. These substances may be used alone or in combination. Possible nitrogen sources may include inorganic nitrogen sources such as ammonia, ammonium sulfate, ammonium chloride, ammonium acetate, ammonium phosphate, ammonium carbonate and ammonium nitrate; amino acids such as glutamic acid, methionine, and glutamine; and organic nitrogen sources such as peptone, NZ-amine, meat extract, yeast extract, malt extract, corn steep liquor, casein hydrolysates, fish or decomposition products thereof, and defatted soybean cake or decomposition products thereof. These nitrogen sources may be used alone or in combination. The medium may include potassium dihydrogen phosphate, dipotassium hydrogen phosphate or the corresponding sodium-containing salts as phosphorus sources. Possible phosphorus sources may include potassium dihydrogen phosphate, dipotassium hydrogen phosphate or the corresponding sodium-containing salts. Further, inorganic compounds such as sodium chloride, calcium chloride, iron chloride, magnesium

sulfate, iron sulfate, manganese sulfate and calcium carbonate may be used. In addition to the above substances, essential growth substances, such as amino acids and vitamins, may be included. Appropriate precursors may be also added to the culture media. The above-mentioned substances may be suitably added to the culture medium in batch, fed-batch or continuous mode during cultivation, but are not particularly limited thereto. The pH of the culture may be adjusted by suitably adding basic compounds such as sodium hydroxide, potassium hydroxide, and ammonia, or acidic compounds such as phosphoric acid and sulfuric acid.

An anti-foaming agent such as fatty acid polyglycol esters may be used to suppress the development of foam. In order to maintain aerobic condition, oxygen or oxygen-containing gas (e.g., air) is introduced into the culture broth. The temperature of the culture broth is normally 27°C to 37°C, specifically 30°C to 35°C. The culture may be continued until the production of D-lactic acid reaches a desired level, and may be normally continued for 10 to 100 hours. D-lactic acid may be released into the culture medium or included within the cells.

Furthermore, recovering D-lactic acid from the culture broth may be performed by a known method known in the art.

Specifically, the known method for recovering D-lactic acid is not particularly limited, as long as the method can recover D- lactic acid in the culture broth. Specifically, centrifugation, filtration, extraction, spraying, drying, evaporation, precipitation, crystallization, electrophoresis, fractional dissolution (e.g., ammonium sulfate precipitation, etc.), or chromatography (e.g., ion exchange chromatography, affinity chromatography, hydrophobic chromatography, and size exclusion chromatography, etc.) may be used.

Hereinafter, the present invention will be described in more detail with reference to Examples. However, these Examples are for illustrative purposes only, and the invention is not intended to be limited by these Examples.

Example 1: Analysis of Fermentation ratio of D- and L-lactic acids of wild-type *LactoBacillus sp.* strain

Each of 10 types of wild-type lactic acid -producing strains was inoculated in 50 ml of GY medium (5% dextrose, 1% yeast extract, 0.05% sodium citrate, 3% $CaCO_3$, 0.02% $MgSO_4$, 0.001% $MnSO_4$, 0.001% $FeSO_4$ and 0.001% NaCl) and then cultured under anaerobic conditions at 37°C for 40 hours, followed by HPLC analysis for a ratio of D-lactic acid and L-lactic acid in the fermentation broth (FIG. 1). FIG. 1 shows a graph representing the results of analyzing a ratio of D-lactic

acid and L-lactic acid which were produced by 10 types of wild-type lactic acid- producing strains.

Lactobacillus paracasei, *Lactobacillus casei* and *Lactobacillus rhamnosus* showing high productivity of lactic acid and much higher ratio of L-lactic acid were selected from 10 types of the strains. , The selected strains were modified to produce D-lactic acid.

Example 2: Comparison of nucleotide sequences of L-lactate dehydrogenase (L-LDH)

To delete a gene inducing overproduction of L-lactic acid in each of the strains selected in Example 1, homology between L-LDH-encoding gene of the selected strains was compared and analyzed by searching U.S. National Center for Biotechnology Information (NCBI) (*www.ncbi.nlm.nih.gov//www.ncbi.nlm.nih.gov*) (Table 1).

[Table 1]

Homology comparison between L-LDH gene of *Lactobacillus paracasei* and homologous genes

Homology with ldh of *Lactobacillus paracasei*	Homology with ldh1 of *Lactobacillus paracasei*
Lactobacillus casei ldh1 100%	*Lactobacillus casei* ldh2 99%
Lactobacillus rhamnosus ldh(LGG_02523) 91%	*Lactobacillus rhamnosus* ldh(LGG_00606) 79%

As shown in Table 1, the L-LDH-encoding genes of 3 types of lactic acid-producing strains have very similar nucleotide sequences to each other, and in particular, ldh1 of *Lactobacillus casei* is known as an important L-lactic acid-producing gene (*J. Ind. Microbiotechnol.*, 2008, 35:579-586). Meanwhile, *ldh2* of *Lactobacillus casei* is another L-lactic acid-producing gene. The ldh1 and ldh2 genes were deleted to prepare a strain producing optically pure D-lactic acid. In summary, from the total 3 types of parent strains, *ldh* and *ldh1* genes of *Lactobacillus paracasei*, *ldh1* and *ldh2* genes of *Lactobacillus casei*, and 2 types of *ldh* genes of *Lactobacillus rhamnosus* were selected as genes for deletion.

Example 3: Construction of L-LDH-deletion/D-LDH-insertion vectors

Vectors for deletions of the L-LDH genes of *Lactobacillus paracasei*, *Lactobacillus casei* and *Lactobacillus rhamnosus*, which were selected in Example 2, were prepared. In order to prepare a cassette for deleting L-LDH and inserting D-LDH at the same time, sequences adjacent to ORF of *ldh* and *ldh1* of *Lactobacillus paracasei*, *ldh1* and *ldh2* of *Lactobacillus casei*, and LGG02523 and LGG00606 of *Lactobacillus rhamnosus* were used as homologous

nucleotide sequence, and primers of SEQ ID NOs. 1 to 24 were prepared (Table 2).

[Table 2]

Nucleotide sequence of primer

SEQ ID NO:	Nucleotide sequence (5'-3')	Template
1	atatgcctcgagcgggatttcctaggccaacaatcat	*Lb. paracasei, Lb.casei*
2	ttgcgtaagcaaaaattttagtcatggtgatatcatcctttcttatgtgc	*Lb. paracasei, Lb.casei*
3	gcacataagaaaggatgatatcaccatgactaaaatttttgcttacgcaa	*Lb.delbrueckii*
4	tggttgcttacttatcagtgatcgtgatgattagccaaccttaactggagtttca	*Lb.delbrueckii*
5	tgaaactccagttaaggttggctaatcatcacgatcactgataagtaagcaacca	*Lb. paracasei, Lb.casei*
6	atatgcactagtgcttgttaaggatttgtgtcaagcctt	*Lb. paracasei, Lb.casei*
7	atctctcgagtctgacttacctttcggatcaaaat	*Lb. paracasei, Lb.casei*
8	ctcaaattcctcctcatgaagatct	*Lb. paracasei, Lb.casei*
9	cgtcaagatcttcatgaggaggaatttgagatgaaaattattgcatatgc	*Lb. plantarum*
10	ccgttaagctgagcgcttaacctgacgagcttagtcaaacttaacttgcg	*Lb. plantarum*
11	gctcgtcaggttaagcgctcagctt	*Lb. paracasei,*

		Lb.casei
12	atatactagtccgttggctgggcattgcgtcattc	*Lb. paracasei*, *Lb.casei*
13	ccccctcgagctggtaatacatcattaactgccgc	*Lb.rhamnosus*
14	ttgcgtaagcaaaaattttagtcatggtgatatcatcctttcttatgtgc	*Lb.rhamnosus*
15	gcacataagaaaggatgatatcaccatgactaaaatttttgcttacgcaa	*Lb.delbrueckii*
16	ggtttaaaatcagttatggtgaagattagccaaccttaactggagtttca	*Lb.delbrueckii*
17	tgaaactccagttaaggttggctaatcttcaccataactgattttaaacc	*Lb.rhamnosus*
18	tagaactagtttattcagcacttgagtaagtcctt	*Lb.rhamnosus*
19	ccccctcgagaaccaagcgtccaagaatgtttgct	*Lb.rhamnosus*
20	gtacagcatatgcaataattttcatcctaaacccctccttgacaggtagc	*Lb.rhamnosus*
21	gctacctgtcaaggaggggtttaggatgaaaattattgcatatgctgtac	*Lb. plantarum*
22	aaaaatactgacgatgggttgtgttttagtcaaacttaacttgcgtatca	*Lb. plantarum*
23	tgatacgcaagttaagtttgactaaaacacaacccatcgtcagtattttt	*Lb.rhamnosus*
24	tagaactagtcaaccgttgtcgaaagcattgcggt	*Lb.rhamnosus*

The sequence of 700 base pairs at 5′ region (ldh.pc_UP_700) and the sequence of 700 base pairs at 3′ region (ldh.pc_DOWN_700) of *ldh* gene ORF were amplified using the genome of *Lactobacillus paracasei* as a template and primers of SEQ ID NOS. 1 and 2, and primers of SEQ ID NOS: 5 and 6. The sequence of 700 base pairs at 5′ region (ldh1.pc_UP_700) and the sequence of 700 base pairs at 3′ region (ldh1.pc_DOWN_700) of *ldh1* gene ORF were also

amplified using primers of SEQ ID NOS. 7 and 8, and primers of SEQ ID NOS: 11 and 12.

Meanwhile, to amplify the D-LDH gene, DNA fragments of *ldhA*(*Lb. db*) and *ldhD*(*Lb. pl*) were prepared using the genomes of *Lactobacillus delbrueckii* and *Lactobacillus plantarum* as a template and primers of SEQ ID NOS. 3 and 4, and primers of SEQ ID NOS: 9 and 10.

Subsequently, An overlapping PCR was conducted using the amplified DNA fragments, ldh.pc_UP_700, ldh.pc_DOWN_700 and *ldhA*(*Lb. db*), and primers of SEQ ID NOS. 1 and 6 so as to prepare a δldh.pc-ldhA(*Lb. db*) cassette. The δldh.pc-ldhA(*Lb. db*) cassette has a nucleotide sequence homologous to the sequences adjacent to *ldh* ORF region and D-lactate dehydrogenase is located in the middle of the cassette. Further, *ldh1* gene was subjected to the same procedures to prepare a δldh1.pc-ldhD(*Lb. pl*) cassette. In this regard, each cassette was designed to contain *Xho*I restriction enzyme site at 5'-end, and *Spe*I restriction enzyme site at 3'-end.

Because *ldh1* and *ldh2* genes of *Lactobacillus casei* were very similar to *ldh* and *ldh1* genes of *Lactobacillus paracasei*, respectively, the same primers were used. ldh1.ca_UP_700 and the sequence of 700 base pairs at 3' region (ldh1.ca_DOWN_700) were amplified using the genome of *Lactobacillus casei* as a template and primers of SEQ ID NOs.

1 and 2, and primers of SEQ ID NOS: 5 and 6. the 700 base pairs at 5′ region(ldh2.ca_UP_700) and the sequence of 700 base pairs at 3′ region (ldh2.ca_DOWN_700) of *ldh2* gene ORF were also amplified using primers of SEQ ID NOS. 7 and 8, and primers of SEQ ID NOS: 11 and 12.

Subsequently, An overlapping PCR was conducted using ldh1.ca_UP_700, ldh1.ca_DOWN_700 ,ldhA(*Lb. db*) and primers of SEQ ID NOS. 1 and 6 so as to prepare a δldh1.ca-ldhA(*Lb. db*) cassette. The δldh1.ca-ldhA(*Lb. db*) cassette has a nucleotide sequence homologous to the sequences adjacent to *ldh1* ORF region and D-lactate dehydrogenase is located in the middle of the cassette. Further, *ldh2* gene was subjected to the same procedures to prepare a δldh2.ca-ldhD(*Lb. pl*) cassette.

The sequence of 700 base pairs at 5′ region (LGG_02523_UP_700) and the sequence of 700 base pairs at 3′ region (LGG_02523 _DOWN_700) of LGG_02523 gene ORF were amplified using the genome of *Lactobacillus rhamnosus* as a template and primers of SEQ ID NOS. 13 and 14, and primers of SEQ ID NOS: 17 and 18. The sequence of 700 base pairs at 5′ region (LGG_00606_UP_700) and the sequence of 700 base pairs at 3′ region (LGG_00606_DOWN_700) of LGG_00606 gene ORF were also amplified using primers of SEQ ID NOS. 19 and 20, and primers of SEQ ID NOS: 23 and 24.

Meanwhile, to amplify the D-LDH gene, DNA fragments of *ldhA*(*Lb. db*) and *ldhD*(*Lb. pl*) were prepared using the genomes of *Lactobacillus delbrueckii* and *Lactobacillus plantarum* as a template and primers of SEQ ID NOS. 15 and 16, and primers of SEQ ID NOS: 21 and 22.

Subsequently, An overlapping PCR was conducted using the amplified DNA fragments, LGG_02523_UP_700, LGG_02523 _DOWN_700, *ldhA*(*Lb. db*) and primers of SEQ ID NOs. 13 and 18 so as to prepare a δLGG_02523-*ldhA*(*Lb. db*) cassette. The δLGG_02523-*ldhA*(*Lb. db*) cassette has a nucleotide sequence homologous to LGG_02523 ORF region and D-lactate dehydrogenase is located in the middle of the cassette. Further, LGG_00606 gene was subjected to the same procedures to prepare a δLGG_00606-*ldhD*(*Lb. pl*) cassette. In this regard, each cassette was designed to contain XhoI restriction enzyme site at 5'-end, and *Spe*I restriction enzyme site at 3'-end.

Subsequently, each of 6 types of the cassettes was cloned using *Xho*I and *Spe*I restriction enzyme sites into a heat-sensitive vector, pG+host6 which is characterized in that it contains ampicillin- and erythromycin-resistant genes and thus is used as a shuttle vector of *E. coli*-Lactic acid bacteria, and it is not amplified in *Lactobacillus* at 42°C. Therefore, 6 types of vectors, *pG+host6-δldh.pc-ldhA*(*Lb. db*) and *pG+host6-δldh1.pc-ldhD*(*Lb. pl*), *pG+host6-*

δ1dh1.ca-1dhA(*Lb. db*) and *pG+host6-δ1dh2.ca-1dhD*(*Lb. pl*), and *pG+host6-δLGG_02523-1dhA*(*Lb. db*) and *pG+host6-δLGG_00606-1dhD*(*Lb. pl*) were prepared.

Example 4: Preparation of transformants

Lactobacillus paracasei, *Lactobacillus casei* or *Lactobacillus rhamnosus* strains cultured in MRS solid media for one day were inoculated in 10 ml of MRS media, followed by stationary culture at 37°C for one day. 50 ml of MRS was put in 50 ml of tube, and 500 μl of each strain cultured for one day was inoculated thereto, followed by stationary culture at 37°C for 3 hours and 30 minutes. When OD600 reached 0.8, the culture broths were placed in an ice bath for 5 minutes. Thereafter, the media were removed from the culture broths by centrifugation to obtain the only strains. The strains were washed with a washing buffer (5 mM sodium phosphate, 1 mM $MgCl_2$, pH 7.4) twice. Subsequently, 25 μl of 0.5 M sucrose solution was added to the strains, followed by suspension. Each 50 μl thereof was dispensed. Each 200 ng of the vectors prepared in Examples 3 were added to the strains, followed by electroporation under the conditions of 1800 v, 25 F and 200 Ω. Thereafter, the strains were cultured in 500 μl of MRS at 37°C for 2 hours, and then spread on MRS solid media (MRSE) containing 10 μg/ml of

erythromycin, and cultured at 30°C for 3 days to obtain colonies.

Example 5: Preparation of D-ldh-inserted strain

A portion of the colonies obtained from the transformant derived from *Lactobacillus paracasei* (*Lactobacillus* strain introduced with a *pG+host6-δ1dh1-ldhA* plasmid containing *Lactobacillus delbrueckii*-derived *ldhA*) among the colonies obtained in Example 4 was inoculated in 1 ml of liquid MRS media containing 10 μg/ml of erythromycin, followed by stationary culture at 42°C for one day for induction of primary crossover. 100 μl of the culture broth was spread on solid MRSE media, and incubated for 7 days to obtain colonies. Each single colony was subcultured on solid MRSE media at 42°C for 2 days. Each of the obtained strains was inoculated in 1.5 ml tube containing 1 ml of MRS, followed by stationary culture at 37°C for one day for induction of secondary crossover. A portion of the strain cultured at 37°C was subjected to colony PCR to examine insertion of *ldhA*(*Lb. db*) gene at *ldh1* region, and single colonies were selected on solid MRS media. Single colonies were subjected to PCR to examine deletion of *ldh* gene and insertion of D-lactate dehydrogenase, and finally, a *ldh1*::*ldhA*(*Lb. db*) strain was prepared. This strain was used as a parent strain, and *ldh* deletion and *ldhD*(*Lb. pl*)

insertion were conducted in the same manner to prepare a final D-lactic acid-producing strain, in which two types of L-lactate dehydrogenase were deleted.

Meanwhile, *Lactobacillus casei* and *Lactobacillus rhamnosus* were subjected to the same procedures to prepare D-lactic acid-producing strains, in which two types of L-lactate dehydrogenase were deleted.

Comparative Example 1: Test of lactic acid fermentation of novel modified D-type Lactobacillus

Fermentation results between the novel modified D-type Lactobacillus which was prepared in the present invention and 2 types of the known recombinant D-lactic acid-producing strains based on *Lb. plantarum*. In this regard, the known recombinant D-lactic acid-producing strain based on *Lb. plantarum* is a strain in which any one or both of 2 types of its own L-lactate dehydrogenase was/were deleted, and it was prepared by a similar method of the paper published by Okano et al. (*Appl. Environ. Microbiol.* (2009) 462~467). These strains have *ldhL1* or *ldhL2* gene deletion, and produce D-lactic acid with optical purity of 99% or higher.

In a specific comparative experiment, 2 types of *Lactobacillus plantarum*-based D-lactic acid-producing strains and novel 3 types of recombinant Lactobacillus

strains were cultured on solid MRS media for one day, and each one loop of the obtained cells was inoculated in liquid MRS, followed by culture at 37°C for one day. Total 5 types of recombinant *Lactobacillus* strains were inoculated in 250 ml-baffle flask containing 25 ml of GY liquid media with initial cell optical density of 0.1 at 600 nm. The experiment was carried out in an incubator at a temperature of 37°C with shaking at 100 rpm. Total culture time was 42 hours. The culture broth samples were collected at an initial inoculation time and a final fermentation time, and a proper amount thereof was centrifuged to obtain the supernatant, followed by HPLC. As a result, the initial glucose concentration was 53 g/l. The data analyzed were the average of the results from the experiments repeated twice. The results are summarized in the following Table 3. Enzymatic quantification showed that lactic acid produced in all the samples was optically pure D-lactic acid (Lactic acid, R-Biopharm, Germany).

[Table 3]

Comparison of lactic acid productivity between strains

Strain	Yield (%)	Productivity (g/l·h)	Sugar Consumption (g/l)	L-lactic acid	D-lactic acid

				(g/l)	(g/l)
Lb.plantarum ΔldhL1	81	0.81	42	0	34
Lb.plantarum ΔldhL1 ΔldhL2	75	0.69	39	0	29
Lb.paracasei ldh::ldhA ldh1::ldhD	93	1.2	53	0	49
Lb.casei ldh1::ldhA ldh2::ldhD	89	1.1	53	0	47
Lb.rhamnosus LGG_02523::ldhA LGG_00606::ldhD	85	0.99	49	0	42

As shown in Table 3, it was found that 3 types of the novel recombinant *Lactobacillus* strains prepared in the present invention had higher yield, productivity and production amount of D-lactic acid than the known strains (*Lb. plantarum ΔldhL1* and *Lb. plantarum ΔldhL1 ΔldhL2*) in which any one or both of 2 types of their own L-lactate dehydrogenase was/were deleted. In particular, it was found that the transformant derived from *Lactobacillus paracasei* (*Lb. paracasei ldh::ldhA ldh1::ldhD*) had the highest yield, productivity and production amount of D-lactic acid.

Accordingly, the present inventors designated the transformant (*Lb. paracasei ldh::ldhA ldh1::ldhD*) as *Lactobacillus paracasei* CC02-0095. The transformant, which was modified by substituting the polynucleotide encoding LDH (SEQ ID NO: 25) and the polynucleotide encoding LDH1 (SEQ ID NO: 26) of *Lactobacillus paracasei* with the polynucleotide

encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and the polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum,* was the most excellent in terms of yield, productivity, and production amount of the D-lactic acid and. The transformant was deposited with the Korean Culture Center of Microorganisms (hereinafter, abbreviated to "KCCM", under the Budapest Treaty) on April 2, 2012 under Accession No. KCCM11273P.

It will be apparent to those skilled in the art that various modifications and changes may be made without departing from the scope and spirit of the invention. Therefore, it should be understood that the above embodiment is not limitative, but illustrative in all aspects. The scope of the invention includes not only the appended claims but alsoe all changes and modifications of metes and bounds of the claims, or equivalents..

Effect of the invention

The D-lactic acid-producing strain of the present invention is prepared from an L-lactic acid-producing strain having excellent lactic acid productivity, and thus it has excellent D-lactic acid productivity. Therefore, the strain can be widely used to improve the productivity of various

products which are manufactured using D-lactic acid as a raw material.

WHAT IS CLAIMED IS:

1. A D-lactic acid-producing strain modified to attenuate or inactivate L-lactate dehydrogenase (L-LDH) activity and to enhance D-lactate dehydrogenase (D-LDH) activity.

2. The strain according to claim 1, wherein an L-LDH-encoding polynucleotide is inactivated.

3. The strain according to claim 2, wherein the L-LDH-encoding polynucleotide is selected from the group consisting of *ldh* (SEQ ID NO: 25) and *ldh1* (SEQ ID NO: 26)of Lactobacillus paracasei, *ldh1* (SEQ ID NO: 27) and *ldh2* (SEQ ID NO: 28)of Lactobacillus paracasei, *ldh*(*LGG_02523*) (SEQ ID NO: 29) and *ldh* (*LGG_00606*)(SEQ ID NO: 30)of Lactobacillus rhamnosus.

4. The strain according to claim 1, wherein a D-LDH-encoding polynucleotide is introduced.

5. The strain according to claim 4, wherein the D-LDH-encoding polynucleotide is derived from *Lactobacillus plantarum* or *Lactobacillus delbrueckii*.

6. The strain according to claim 5, wherein the D-LDH-encoding polynucleotide is a LDHA (SEQ ID NO: 31)-encoding polynucleotide of Lactobacillus delbrueckii or a LDHD (SEQ ID NO: 32)-encoding polynucleotide of Lactobacillus *plantarum*.

7. The strain according to claim 1, wherein one or more of heterogenous D-LDH-encoding polynucleotides are substituted for the L-LDH-encoding polynucleotide in the chromosome and are overexpressed.

8. The strain according to claim 1, wherein the strain before modification is a strain producing L- lactic acid.

9. The strain according to claim 8, wherein the strain is a *Lactobacillus sp* strain.

10. The strain according to claim 8, wherein a polynucleotide encoding LDH1 (SEQ ID NO: 27) and a polynucleotide encoding LDH2 (SEQ ID NO: 28) of *Lactobacillus casei* are substituted with a polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and a polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*, respectively.

11. The strain according to claim 8, wherein a polynucleotide encoding LDH (SEQ ID NO: 25) and a polynucleotide encoding LDH1 (SEQ ID NO: 26) of *Lactobacillus paracasei* are substituted with a polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and a polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*, respectively.

12. The strain according to claim 8, wherein a polynucleotide encoding LDH(LGG_02523) (SEQ ID NO: 29) and a polynucleotide encoding LDH(LGG_00606) (SEQ ID NO: 30) of *Lactobacillus rhamnosus* are substituted with a polynucleotide encoding LDHA (SEQ ID NO: 31) of *Lactobacillus delbrueckii* and a polynucleotide encoding LDHD (SEQ ID NO: 32) of *Lactobacillus plantarum*, respectively.

13. The strain according to claim 12, wherein it is *Lactobacillus paracasei* CC02-0095 deposieted under the Accession No. KCCM11273P.

14. A method for preparing a modified D-lactic acid-producing strain, comprising:

(a) attenuating or inactivating L-lactate dehydrogenase (L-LDH) activity in an L-lactic acid-producing strain to obtain a modified lactic acid-producing strain; and

(b) introducing or enhancing D-lactate dehydrogenase (D-LDH) activity in the modified lactic acid-producing strain.

15. The method according to claim 14, wherein the L-lactic acid-producing strain is a *Lactobacillus sp.* strain.

16. The method according to claim 14, wherein the L-lactic acid-producing strain is selected from the group consisting of *Lactobacillus brevis*, *Lactobacillus pentosus*, *Lactobacillus rhamnosus*, *Lactobacillus jensenii*, *Lactobacillus plantarum*, *Lactobacillus paraplantarum*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, and *Lactobacillus casei*.

17. The method according to claim 14, wherein the L-LDH activity is attenuated or inactivated by substitution, deletion, insertion or addition of an L-LDH-encoding polynucleotide.

18. The method according to claim 17, wherein the L-LDH-encoding polynucleotide is selected from the group consisting of *ldh* (SEQ ID NO: 25) and *ldh1* (SEQ ID NO: 26) of Lactobacillus paracasei, *ldh1* (SEQ ID NO: 27) and *ldh2* (SEQ ID NO: 28) of Lactobacillus casei, *ldh(LGG_02523)* (SEQ ID NO: 29) and *ldh(LGG_00606)* (SEQ ID NO: 30) of Lactobacillus rhamnosus.

19. The method according to claim 14, wherein the D-LDH activity is introduced or enhanced by introducing a D-LDH-encoding polynucleotide into the chromosome of the modified lactic acid-producing strain.

20. The method according to claim 14, wherein the D-LDH activity is introduced or enhanced by introducing an expression vector including a D-LDH-encoding polynucleotide into the modified lactic acid-producing strain.

21. The method according to claim 19 or 20, wherein the D-LDH-encoding polynucleotide is a polynucleotide encoding LDHA (SEQ ID NO: 31) of Lactobacillus delbrueckii or a polynucleotide encoding a polypeptide of *Lactobacillus plantarum* (SEQ ID NO: 32).

22. A method for producing D-lactic acid, comprising:

(a) culturing the modified D-lactic acid-producing strain of any one of claims 1 to 13 to obtain a culture broth; and

(b) recovering D-lactic acid from the culture broth.

23. The method according to claim 22, wherein the modified D-lactic acid-producing strain is *Lactobacillus paracasei* CC02-0095deposieted under the KCCM11273P.

24. The method according to claim 22, wherein the D-lactic acid is recoverd by a method selected from the group consisting of centrifugation, filtration, extraction, spraying, drying, evaporation, precipitation, crystallization, electrophoresis, fractional dissolution, chromatography and combinations thereof.