associated D-Amino Acid Oxidase from Candida tropicalis", Agricul. and Biol. Chemistry, 1986, Vol. 50, No. 10, page 2637

Non-Patent Reference 2: "Characterization and High-level Production of D-Amino Acid Oxidase in Candida boidinii", Biosci. Biotechnol. Biochem., 2001, Vol. 65, No. 3, page 627

Non-Patent Reference 3: "Production of D-Amino Acid Oxidase by Candida tropicalis", J. Ferment. Technol., 1977, Vol. 55, No. 1, page 13

Non-Patent Reference 4: "Enzymatic Conversion of Racemic Methionine to the L-Enantiomer", J. Chem. Soc. Chem. Commun., 1990, Vol. 13, page 947

Non-Patent Reference 5: "Enzymatic synthesis of chiral intermediates for Omapatrilat, an antihypertensive drug", Biomolecular Engineering, 2001, Vol. 17, page 167

Disclosure ~~of the Invention~~

Problems to be Solved ~~by the Invention~~

【0009】

D-amino acid oxidase obtained in the present ~~invention~~disclosure is an enzyme that oxidizes D-amino acid in the presence of oxygen, and produces hydrogen peroxide, 2-oxo acid and ammonia. Moreover, it is a useful enzyme utilizable for a stereo inversion reaction by which racemic amino acid is quantitatively converted to L-amino acid via keto acid or imino acid, by using D-amino acid oxidase and amino acid dehydrogenase, or amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity.

【0010】

An object of the present embodiments disclosed herein~~invention~~ is to provide novel a D-amino acid oxidase. In addition, an object of the present ~~invention~~disclosure is to clarify the amino acid sequence of the D-amino acid oxidase and the base sequence of the gene, and provide a microorganism or

transformant having an ability to produce the enzyme.

【0011】

In addition, the object of a preferable embodiment of the present ~~invention~~disclosure is to provide a transformant that highly produces D-amino acid oxidase and simultaneously produces amino acid dehydrogenase, and an enzyme having a coenzyme-regenerating activity.

【0012】

Moreover, the present ~~invention~~disclosure provides an efficient production method of L-amino acid, 2-oxo acid or cyclic imine, which utilizes the above D-amino acid oxidase or the above transformant.

Means of Solving the Problems

【0013】

In view of the above-mentioned problems, the present inventors isolated and purified D-amino acid oxidase from Candida intermedia, achieved isolation of D-amino acid oxidase gene and expression in a host microorganism, and prepared a transformant having high activity. In the case of D-amino acid oxidase obtained in the present ~~invention~~disclosure, high production was possible even when a transformant was cultured without using a means such as suppression of expression, addition of an inducer and the like. By reacting, with the racemic amino acid, D-amino acid oxidase obtained in the present invention, solely or in combination with an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity, production of 2-oxo acid or L-amino acid has been enabled, which resulted in the completion of the present ~~invention~~disclosure.

【0014】

The present inventors have further conducted intensive studies and prepared a transformant having a D-amino acid oxidase gene, and respective genes encoding amino acid dehydrogenase and an enzyme having a

coenzyme-regenerating activity. In general, a transformant having plural and different enzyme genes is expected to show decreased prouction volume of each enzyme, as compared to transformants independently having respective enzyme genes. Surprisingly, however, they have found that, in a preferable embodiment of the present ~~invention~~disclosure, a transformant having a D-amino acid oxidase gene, and genes encoding amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity shows an increased activity value for one culture medium of D-amino acid oxidase by not less than 50-fold as compared to a transformant having a D-amino acid oxidase gene alone (corresponding to Example 12 mentioned below).

【0015】

Heretofore, with regard to a transformant having a D-amino acid oxidase gene and one or more genes of polypeptide having a different enzyme activity from that of D-amino acid oxidase (e.g., amino acid dehydrogenase, and coenzyme-regenerating enzyme), an embodiment wherein the activity of D-amino acid oxidase markedly increased as compared to a transformant having a D-amino acid oxidase gene alone is not known. For example, the aforementioned Patent Reference 6 (WO05/090950) does not disclose an embodiment wherein the activity of D-amino acid oxidase of a transformant having genes of D-amino acid oxidase, amino acid dehydrogenase, and an enzyme having a coenzyme-regenerating activity increased as compared to a transformant having a D-amino acid oxidase gene alone.

【0016】

The fact that the above-mentioned transformant having respective genes shows a remarkably improved D-amino acid oxidase activity as compared to a transformant having a D-amino acid oxidase gene alone

was found by a special attempt in the present inventor. Moreover, an efficient production of L-amino acid has become possible by reacting a transformant having genes of D-amino acid oxidase, amino acid dehydrogenase and an enzyme having coenzyme-regenerating activity, which is obtained in a preferable embodiment of the present invention, with racemic amino acid, which resulted in the completion of the present ~~invention~~disclosure.

【0017】

Accordingly, the present ~~invention~~disclosure has the following one or plural characteristics.

[1] One of the characteristics of the present ~~invention~~disclosure is a polypeptide derived from Candida intermedia and having a D-amino acid oxidase activity, which has the following physicochemical properties:

(1) molecular weight

molecular weight about 252,000

molecular weight of subunit about 42,000;

(2) range of operative temperature

temperature range at least 20°C - 60°C;

(3) range of operative pH

pH range at least 6 - 10.5.

[2] One of the characteristics of the present ~~invention~~disclosure is a polypeptide of the following (a), (b) or (c):

(a) a polypeptide having the amino acid sequence shown in Sequence Listing SEQ ID NO: 1;

(b) a polypeptide having an amino acid sequence wherein one or a few amino acids of the amino acid sequence shown in Sequence Listing SEQ ID NO: 1 are substituted, inserted, deleted and/or added, and having a D-amino acid oxidase activity;

(c) a polypeptide having an amino acid sequence having a homology of not less than 69% with the amino acid sequence shown in Sequence Listing SEQ ID NO: 1, and having a D-amino acid oxidase activity.

[3] One of the characteristics of the present invention is a DNA encoding the polypeptide of the aforementioned [1] or [2].

[4] The DNA of preferable embodiments of the present ~~invention~~disclosure is a DNA of the following (d), (e) or (f):

(d) a DNA having a base sequence shown in Sequence Listing SEQ ID NO: 2;

(e) a DNA having a base sequence wherein one or a few bases of the base sequence shown in Sequence Listing SEQ ID NO: 2 are substituted, inserted, deleted and/or added, and encoding a polypeptide having a D-amino acid oxidase activity;

(f) a DNA having a base sequence having a homology of not less than 67% with the base sequence shown in Sequence Listing SEQ ID NO: 2, and encoding a polypeptide having a D-amino acid oxidase activity.

[4a] In one aspect, there is provided a polypeptide of the following (a), (b) or (c):

(a) a polypeptide having an amino acid sequence shown in Sequence Listing SEQ ID NO: 1;

(b) a polypeptide having an amino acid sequence wherein one or a few amino acids of the amino acid sequence shown in Sequence Listing SEQ ID NO: 1 are substituted, inserted, deleted and/or added, and having a D-amino acid oxidase activity;

(c) a polypeptide having an amino acid sequence showing a homology of not less than 90% with the amino acid sequence shown in Sequence Listing SEQ ID NO: 1, and having a D-amino acid oxidase activity.

[4b] In another aspect, there is provided a DNA encoding the polypeptide as defined above.

[4c] In another aspect, there is provided a DNA of the following (d), (e) or (f):

(d) a DNA having a base sequence shown in Sequence Listing SEQ ID NO: 2;

(e) a DNA having a base sequence wherein one or a few bases of the base sequence shown in Sequence Listing SEQ ID NO: 2 are substituted, inserted, deleted and/or added, and encoding a polypeptide having a D-amino acid oxidase activity;

(f) a DNA having a base sequence showing a homology of not less than 90% with the base sequence shown in Sequence Listing SEQ ID NO: 2, and encoding a polypeptide having a D-amino acid oxidase activity.

[5] One of the characteristics of the present ~~invention~~disclosure is a recombinant plasmid obtained by inserting the DNA of the aforementioned [3] or [4] into a vector.

[6] The recombinant plasmid of a preferable embodiment of the present ~~invention~~disclosure is a recombinant plasmid wherein a DNA encoding a polypeptide having a different enzyme activity from D-amino acid oxidase is additionally inserted into a vector.

[7] Another aspect~~One of the characteristics~~ of the present ~~invention~~disclosure is a transformant obtained by transforming a host microorganism with the recombinant plasmid of the aforementioned [5].

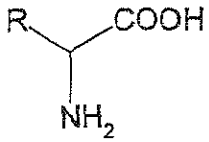
[8] A preferable embodiment of the present ~~invention~~disclosure is the transformant of [7], into which a DNA encoding a polypeptide having an enzyme activity other than that of D-amino acid oxidase has been additionally introduced.

[9] Another aspect~~One of the characteristics~~ of the present ~~invention~~disclosure is a method of producing D-amino acid oxidase, comprising culturing a microorganism having an ability

to produce the polypeptide of the aforementioned [1] or [2], accumulating the polypeptide in the culture, and recovering same.

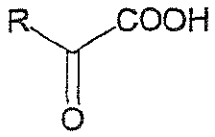
[9a] In another aspect, there is provided a method of producing D-amino acid oxidase, comprising culturing a microorganism having an ability to produce the polypeptide as defined above, accumulating the polypeptide in the culture, and harvesting the polypeptide.

[10] Another aspect~~One of the characteristics~~ of the present ~~invention~~disclosure is a method of producing L-amino acid represented by the formula (2):



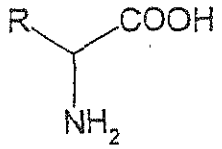
(2)

wherein R represents a C1-20 alkyl group optionally having substituent(s), a C7-20 aralkyl group optionally having substituent(s), or a C6-20 aryl group optionally having substituent(s), or 2-oxo acid represented by the formula (3):



(3)

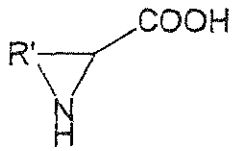
wherein R represents as defined above, which comprises reacting the polypeptide ~~of the foremention [1] or~~ as defined above~~[2]~~, or a microorganism having an ability to produce the polypeptide, with a racemic amino acid represented by the formula (1):



(1)

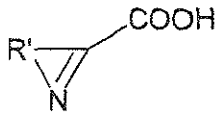
wherein R represents as defined above.

[11] Another aspect~~One of the characteristics~~ of the present ~~invention~~disclosure is a method of producing cyclic L-amino acid represented by the formula (5):



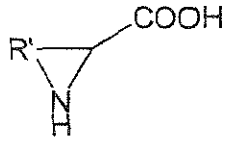
(5)

wherein R′ is a C2-7 alkyl chain optionally having substituent(s), or a cyclic imine represented by the formula (6):



(6)

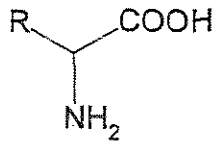
wherein R′ is as defined above, which comprises reacting the polypeptide as defined above~~of [1] or [2]~~, or a microorganism having an ability to produce the polypeptide with a cyclic amino acid represented by the formula (4):



(4)

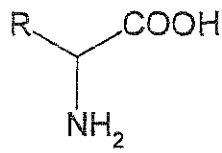
wherein R′ is as defined above.

[12] Another aspect~~One of the characteristics~~ of the present ~~invention~~disclosure is a method of producing L-amino acid represented by the formula (2):



(2)

wherein R represents a C1-20 alkyl group optionally having substituent(s), a C7-20 aralkyl group optionally having substituent(s), or a C6-20 aryl group optionally having substituent(s), which comprises reacting, in the presence of an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity, the polypeptide as defined above~~of [1] or [2]~~, or a microorganism having an ability to produce the polypeptide with a racemic amino acid represented by the formula (1):

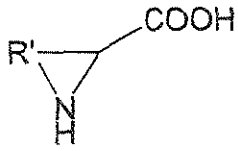


(1)

wherein R represents as defined above.

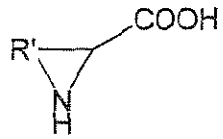
[13] Another aspect~~One of the characteristics~~ of the present ~~invention~~disclosure

is a method of producing cyclic L-amino acid represented by the formula (5):



(5)

wherein R' is a C2-7 alkyl chain optionally having substituent(s), which comprises reacting, in the presence of an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity, the polypeptide as defined above~~of [1] or [2]~~ or a microorganism having an ability to produce the polypeptide with a cyclic amino acid represented by the formula (4):



(4)

wherein R' is as defined above.

[14] One of the characteristics of the present ~~invention~~disclosure is a method of increasing a production amount of D-amino acid oxidase, which comprises transforming a host microorganism with a DNA encoding D-amino acid oxidase and a DNA encoding at least one kind of polypeptide having an enzyme activity different from that of the D-amino acid oxidase.

[15] One of the characteristics of the present ~~invention~~disclosure is a recombinant plasmid comprising a DNA encoding a polypeptide having a D-amino acid oxidase activity and a DNA encoding a polypeptide having an enzyme activity different from that of the D-amino acid oxidase as insertion fragments, which plasmid shows an improved production amount of D-amino acid oxidase as compared to a recombinant plasmid containing only a DNA encoding a polypeptide having the aforementioned D-amino acid oxidase activity as an insertion fragment.

[16] One of the characteristics of the present ~~invention~~disclosure

is a transformant obtained by transforming a host microorganism with a DNA encoding a polypeptide having a D-amino acid oxidase activity and a DNA encoding a polypeptide having an enzyme activity different from that of the D-amino acid oxidase, which transformant shows an improved D-amino acid oxidase activity as compared to a transformant transformed with only a DNA encoding a polypeptide having the aforementioned D-amino acid oxidase activity.

[17] One of the characteristics of the present ~~invention~~disclosure is a method of producing D-amino acid oxidase, which comprises culturing the transformant of [16], accumulating D-amino acid oxidase in a culture, and recovering same.

[18] One of the characteristics of the present ~~invention~~disclosure is a method of producing the aforementioned L-amino acid represented by the formula (2) or the aforementioned cyclic L-amino acid represented by the formula (5), which comprises reacting the transformant of [16] with the aforementioned racemic amino acid represented by the formula (1) or the aforementioned cyclic amino acid represented by the formula (4).

【0038】

Other characteristics and the effects thereof of the present ~~invention~~disclosure can be clarified by the following embodiments and drawings.

Effects of the Invention

【0039】

The present invention has the aforementioned constitution, and can produce novel D-amino acid oxidase efficiently. Moreover, L-amino acid, 2-oxo acid, or cyclic imine can be efficiently produced by utilizing the D-amino acid oxidase, or a transformant or microorganism that produces the D-amino acid oxidase.

【0040】

【1】 A polypeptide having D-amino acid oxidase activity derived from Candida intermedia and the following physicochemical properties:

(1) molecular weight

molecular weight about 252,000

molecular weight of subunit about 42,000;

(2) range of operative temperature

temperature range at least 20°C - 60°C;

(3) range of operative pH

pH range at least 6 - 10.5.

【2】 The polypeptide of claim 1, which is derived from Candida intermedia NBRC0761 strain.

【3】 A polypeptide of the following (a), (b) or (c):

(a) a polypeptide having an amino acid sequence shown in Sequence Listing SEQ ID NO: 1;

(b) a polypeptide having an amino acid sequence wherein one or a few amino acids of the amino acid sequence shown in Sequence Listing SEQ ID NO: 1 are substituted, inserted, deleted and/or added, and having a D-amino acid oxidase activity;

(c) a polypeptide having an amino acid sequence showing a homology of not less than 69% with the amino acid sequence shown in Sequence Listing SEQ ID NO: 1, and having a D-amino acid oxidase activity.

【4】 A DNA encoding the polypeptide of any one of claims 1 to 3.

【5】 A DNA of the following (d), (e) or (f):

(d) a DNA having a base sequence shown in Sequence Listing SEQ ID NO: 2;

(e) a DNA having a base sequence wherein one or a few bases of the base sequence shown in Sequence Listing SEQ ID NO: 2 are substituted, inserted, deleted and/or added, and encoding a polypeptide having a D-amino acid oxidase activity;

(f) a DNA having a base sequence showing a homology of not less than 67% with the base sequence shown in Sequence Listing SEQ ID NO: 2, and encoding a polypeptide having a D-amino acid oxidase activity.

【6】 A recombinant plasmid obtained by inserting the DNA of claim 4 or 5 into a vector.

【7】 The recombinant plasmid of claim 6, wherein the vector is pUC18, pUC19, pBR322, pACYC184, pSTV28, pSTV29, pSC101, pT7Blue or pUCNT, or a derivative thereof.

【8】 The recombinant plasmid of claim 6, which is pCDA003 identified by a restriction enzyme map of Fig. 1.

【9】 The recombinant plasmid of claim 6, wherein a DNA encoding a polypeptide having an activity different from that of D-amino acid oxidase is further inserted into the vector.

【10】 The recombinant plasmid of claim 9, wherein the DNA encoding a polypeptide having an activity different from that of D-amino acid oxidase is a DNA encoding an amino acid dehydrogenase and/or a DNA encoding an enzyme having a coenzyme-regenerating activity.

【11】 The recombinant plasmid of claim 10, wherein a DNA encoding an amino acid dehydrogenase and a DNA

~~encoding an enzyme having a coenzyme-regenerating~~ activity are inserted as the DNA encoding a polypeptide having an enzyme activity different from that of D-amino acid oxidase.

【12】 The recombinant plasmid of claim 10 or 11, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

【13】 The recombinant plasmid of any one of claims 10 to 12, wherein the enzyme having a coenzyme-regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

【14】 The recombinant plasmid of claim 11, which is pFTLBDA identified by a restriction enzyme map of Fig. 7 or pFTPBDA identified by a restriction enzyme map of Fig. 8.

【15】 A transformant obtained by transforming a host microorganism with the recombinant plasmid of claim 6.

【16】 The transformant of claim 15, wherein the host microorganism is Escherichia coli.

【17】 The transformant of claim 15, wherein the transformant is Escherichia coli HB101 (pCDA003) (FERM BP-10639) or JM109 (pCDA003) (FERM BP-10638).

【18】 The transformant of claim 15, into which a DNA encoding a polypeptide having an enzyme activity other than that of D-amino acid oxidase is further introduced.

【19】 The transformant of claim 18, wherein the DNA encoding a polypeptide having an activity different

from that of D-amino acid oxidase is a DNA encoding an amino acid dehydrogenase and/or a DNA encoding an enzyme having a coenzyme-regenerating activity.

【20】 The transformant of claim 19, into which a DNA encoding an amino acid dehydrogenase and a DNA encoding an enzyme having a coenzyme-regenerating activity are introduced as the DNA encoding a polypeptide having an enzyme activity different from that of D-amino acid oxidase.

【21】 The transformant of claim 19 or 20, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

【22】 The transformant of any one of claims 19 to 21, wherein the enzyme having a coenzyme-regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

【23】 The transformant of any one of claims 18 to 22, which is obtained by transforming a host microorganism with the recombinant plasmid of claim 6 and a recombinant plasmid comprising a DNA encoding a polypeptide having an enzyme activity different from that of the D-amino acid oxidase as an insert.

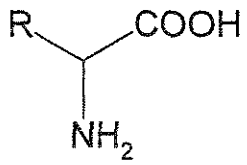
【24】 The transformant of claim 18, which is obtained by transforming a host microorganism with the recombinant plasmid of any one of claims 9 to 14.

【25】 The recombinant plasmid of any one of claims 18 to 24, wherein the host microorganism is Escherichia coli.

~~【26】 A method of producing D-amino acid oxidase,~~ comprising culturing a microorganism having an ability to produce the polypeptide of any one of claims 1 to 3, accumulating the polypeptide in the culture, and harvesting the polypeptide.

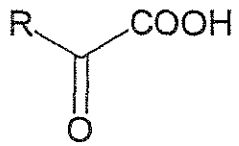
【27】 The production method of claim 26, wherein the microorganism is the transformant of any one of claims 15 to 25 or a microorganism belonging to the genus Candida.

【28】 A method of producing L-amino acids represented by the formula (2):



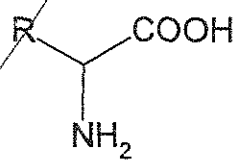
(2)

wherein R represents a C1-20 alkyl group optionally having substituent(s), a C7-20 aralkyl group optionally having substituent(s) or a C6-20 aryl group optionally having substituent(s), or 2-oxo acid represented by the formula (3):



(3)

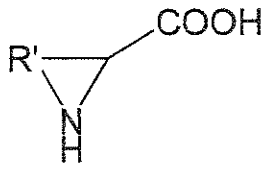
wherein R represents as defined above, which comprises reacting the polypeptide of any one of claims 1 to 3 or a microorganism having an ability to produce the polypeptide, with racemic amino acid represented by the formula (1):



(1)

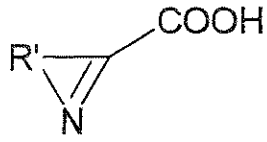
wherein R represents as defined above.

【29】 A method of producing cyclic L-amino acid represented by the formula (5):



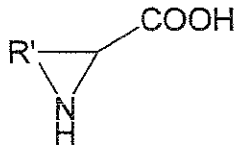
(5)

wherein R' is a C2-7 alkyl chain optionally having substituent(s), or cyclic imine represented by the formula (6):



(6)

wherein R' is as defined above, which comprises reacting the polypeptide of any one of claims 1 to 3 or a microorganism having an ability to produce the polypeptide, with a cyclic amino acid represented by the formula (4):



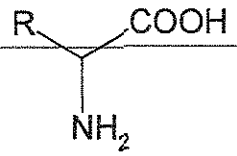
(4)

wherein R' is as defined above.

【30】 The production method of claim 28 or 29, in which the transformant of any one of claims 15 to 17 is used.

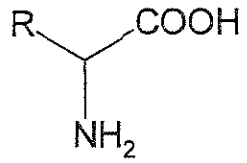
【31】 The production method of any one of claims 28 to 30, wherein the reaction is performed in the presence of catalase.

【32】 A method of producing L-amino acids represented by the formula (2):



(2)

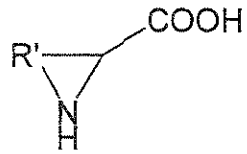
wherein R represents a C1-20 alkyl group optionally having substituent(s), a C7-20 aralkyl group optionally having substituent(s) or a C6-20 aryl group optionally having substituent(s), which comprises reacting, in the presence of an enzyme having an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity, the polypeptide of any one of claims 1 to 3 or a microorganism having an ability to produce the polypeptide with racemic amino acid represented by the formula (1):



(1)

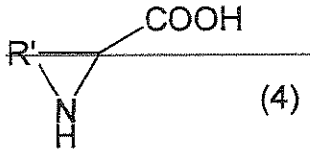
wherein R represents as defined above.

【33】 A method of producing cyclic L-amino acid represented by the formula (5):



(5)

wherein R' is a C2-7 alkyl chain optionally having substituent(s), which comprises reacting, in the presence of an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity, the polypeptide of any one of claims 1 to 3 or a microorganism having an ability to produce the polypeptide with cyclic amino acid represented by the formula (4):



(4)

wherein R' is as defined above.

【34】 The production method of claim 32 or 33, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

【35】 The production method of any one of claims 32 to 34, wherein the enzyme having a coenzyme-regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

【36】 The production method of any one of claims 32 to 35, which comprises use of the transformant of any one of claims 15 to 25.

【37】 The production method of claim 36, which comprises use of a transformant coexpressing 3 enzymes of a D-amino acid oxidase, an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity.

【38】 The production method of claim 37, wherein the transformant is used without disruption of a microbial cell thereof.

【39】 The production method of any one of claims 32 to 38, wherein the reaction is performed in the presence of catalase.

【40】 A method of increasing a production amount of D-amino acid oxidase, which comprises transforming a host microorganism with a DNA encoding D-amino acid oxidase

and a DNA encoding at least one kind of polypeptide having an enzyme activity different from that of the D-amino acid oxidase.

【41】 The method of claim 40, wherein the DNA encoding a polypeptide having an enzyme activity different from that of the D-amino acid oxidase is a DNA encoding amino acid dehydrogenase and/or a DNA encoding an enzyme having a coenzyme-regenerating activity.

【42】 The method of claim 41, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

【43】 The method of claim 42, wherein the leucine dehydrogenase is derived from Bacillus sphaericus NBRC3341 strain.

【44】 The method of claim 42, wherein the phenylalanine dehydrogenase is derived from Bacillus badius IAM11059 strain.

【45】 The method of any one of claims 41 to 44, wherein the enzyme having a coenzyme-regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

【46】 The method of claim 45, wherein the formate dehydrogenase is derived from Thiobacillus sp. KNK65MA strain (FERM BP-7671).

【47】 The method of any one of claims 40 to 46, wherein both a DNA encoding an amino acid dehydrogenase and a DNA encoding an enzyme having a coenzyme-regenerating activity are used for transformation as the DNA encoding a polypeptide having an enzyme activity

~~different from that of D-amino acid oxidase.~~

【48】 The method of any one of claims 40 to 47, wherein the host microorganism is Escherichia coli.

【49】 A recombinant plasmid comprising, as inserts, a DNA encoding a polypeptide having a D-amino acid oxidase activity and a DNA encoding a polypeptide having an enzyme activity different from that of the D-amino acid oxidase, and showing an improved production amount of the D-amino acid oxidase as compared to a recombinant plasmid comprising a DNA encoding the polypeptide having the D-amino acid oxidase activity alone as an insert.

【50】 The recombinant plasmid of claim 49, wherein the DNA encoding the polypeptide having an enzyme activity different from that of the D-amino acid oxidase is a DNA encoding an amino acid dehydrogenase and/or a DNA encoding an enzyme having a coenzyme-regenerating activity.

【51】 The recombinant plasmid of claim 50, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

【52】 The recombinant plasmid of claim 51, wherein the leucine dehydrogenase is derived from Bacillus sphaericus NBRC3341 strain.

【53】 The recombinant plasmid of claim 51, wherein the phenylalanine dehydrogenase is derived from Bacillus badius IAM11059 strain.

【54】 The recombinant plasmid of any one of claims 50

~~to 53, wherein the enzyme having a coenzyme-~~ regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

【55】 The recombinant plasmid of claim 54, wherein the formate dehydrogenase is derived from Thiobacillus sp. KNK65MA strain (FERM BP-7671).

【56】 The recombinant plasmid of any one of claims 49 to 55, into which both a DNA encoding an amino acid dehydrogenase and a DNA encoding an enzyme having a coenzyme-regenerating activity are introduced as the DNA encoding a polypeptide having an enzyme activity different from that of D-amino acid oxidase.

【57】 A transformant obtained by transforming a host microorganism with a DNA encoding a polypeptide having a D-amino acid oxidase activity and a DNA encoding a polypeptide having an enzyme activity different from that of the D-amino acid oxidase, and showing an improved D-amino acid oxidase activity as compared to a transformant transformed with a DNA encoding the polypeptide having the D-amino acid oxidase activity alone.

【58】 The transformant of claim 57, wherein the DNA encoding the polypeptide having an enzyme activity different from that of the D-amino acid oxidase is a DNA encoding an amino acid dehydrogenase and/or a DNA encoding an enzyme having a coenzyme-regenerating activity.

【59】 The transformant of claim 58, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

~~【60】 The transformant of claim 59, wherein the leucine~~ dehydrogenase is derived from Bacillus sphaericus NBRC3341 strain.

【61】 The transformant of claim 59, wherein the phenylalanine dehydrogenase is derived from Bacillus badius IAM11059 strain.

【62】 The transformant of any one of claims 58 to 61, wherein the enzyme having a coenzyme-regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

【63】 The transformant of claim 62, wherein the formate dehydrogenase is derived from Thiobacillus sp. KNK65MA strain (FERM BP-7671).

【64】 The transformant of any one of claims 57 to 63, which is obtained by transforming a host microorganism with a recombinant plasmid comprising a DNA encoding a polypeptide having a D-amino acid oxidase activity as an insert and a recombinant plasmid comprising a DNA encoding a polypeptide having an enzyme activity different from that of the D-amino acid oxidase as insert.

【65】 The transformant of any one of claims 57 to 64, into which both a DNA encoding an amino acid dehydrogenase and a DNA encoding an enzyme having a coenzyme-regenerating activity are introduced as the DNA encoding a polypeptide having an enzyme activity different from that of D-amino acid oxidase.

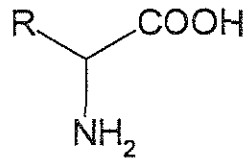
【66】 The transformant of claim 57, which is obtained

by transforming a host microorganism with the recombinant plasmid of any one of claims 49 to 56.

【67】 The transformant of any one of claims 57 to 66, wherein the host microorganism is Escherichia coli.

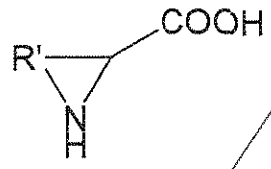
【68】 A method of producing D-amino acid oxidase, comprising culturing the transformant of any one of claims 57 to 67, accumulating D-amino acid oxidase in the culture, and harvesting the D-amino acid oxidase.

【69】 A method of producing L-amino acid represented by the formula (2):



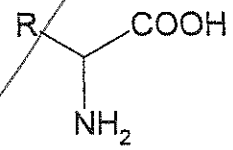
(2)

wherein R represents a C1-20 alkyl group optionally having substituent(s), a C7-20 aralkyl group optionally having substituent(s) or a C6-20 aryl group optionally having substituent(s), or cyclic L-amino acid represented by the formula (5):



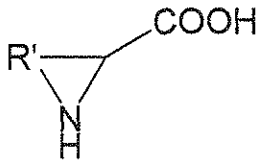
(5)

wherein R' is a C2-7 alkyl chain optionally having substituent(s), which comprises reacting the transformant of any one of claims 57 to 67 with racemic amino acid represented by the formula (1):



(1)

~~wherein R represents as defined above, or cyclic amino~~ acid represented by the formula (4):



(4)

wherein R' is as defined above.

【70】 The production method of claim 69, which comprises using a transformant coexpressing 3 enzymes of a D-amino acid oxidase, an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity.

CLAIMS

1. A polypeptide of the following (a), (b) or (c):
(a) a polypeptide having an amino acid sequence shown in Sequence Listing SEQ ID NO: 1;
(b) a polypeptide having an amino acid sequence wherein one or a few amino acids of the amino acid sequence shown in Sequence Listing SEQ ID NO: 1 are substituted, inserted, deleted and/or added, and having a D-amino acid oxidase activity;
(c) a polypeptide having an amino acid sequence showing a homology of not less than 90% with the amino acid sequence shown in Sequence Listing SEQ ID NO: 1, and having a D-amino acid oxidase activity.

2. A DNA encoding the polypeptide of claim 1.

3. A DNA of the following (d), (e) or (f):
(d) a DNA having a base sequence shown in Sequence Listing SEQ ID NO: 2;
(e) a DNA having a base sequence wherein one or a few bases of the base sequence shown in Sequence Listing SEQ ID NO: 2 are substituted, inserted, deleted and/or added, and encoding a polypeptide having a D-amino acid oxidase activity;
(f) a DNA having a base sequence showing a homology of not less than 90% with the base sequence shown in Sequence Listing SEQ ID NO: 2, and encoding a polypeptide having a D-amino acid oxidase activity.

4. A recombinant plasmid obtained by inserting the DNA of claim 2 or 3 into a vector.

5. The recombinant plasmid of claim 4, which is pCDA003 identified by a restriction enzyme map of Fig. 1.

6. The recombinant plasmid of claim 4, wherein a DNA encoding a polypeptide having an activity different from that of D-amino acid oxidase is further inserted into the vector.

7. The recombinant plasmid of claim 6, wherein the DNA encoding a polypeptide having an activity different from that of D-amino acid oxidase is a DNA encoding an amino acid dehydrogenase and/or a DNA encoding an enzyme having a coenzyme-regenerating activity.

8. The recombinant plasmid of claim 7, wherein a DNA encoding an amino acid dehydrogenase and a DNA encoding an enzyme having a coenzyme-regenerating activity are inserted as the DNA encoding a polypeptide having an enzyme activity different from that of D-amino acid oxidase.

9. The recombinant plasmid of claim 7 or 8, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

10. The recombinant plasmid of any one of claims 7 to 9, wherein the enzyme having a coenzyme-regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

11. The recombinant plasmid of claim 8, which is pFTLBDA identified by a restriction enzyme map of Fig. 7 or pFTPBDA identified by a restriction enzyme map of Fig. 8.

12. A transformant obtained by transforming a host microorganism with the recombinant plasmid of claim 4.

13. The transformant of claim 12, wherein the transformant is Escherichia coli HB101 (pCDA003) (FERM BP-10639) or JM109 (pCDA003) (FERM BP-10638).

14. The transformant of claim 12, into which a DNA encoding a polypeptide having an enzyme activity other than that of D-amino acid oxidase is further introduced.

15. The transformant of claim 14, wherein the DNA encoding a polypeptide having an activity different from that of D-amino acid oxidase is a DNA encoding an amino acid dehydrogenase and/or a DNA encoding an enzyme having a coenzyme-regenerating activity.

16. The transformant of claim 15, into which a DNA encoding an amino acid dehydrogenase and a DNA encoding an enzyme having a coenzyme-regenerating activity are introduced as the DNA encoding a polypeptide having an enzyme activity different from that of D-amino acid oxidase.

17. The transformant of claim 15 or 16, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

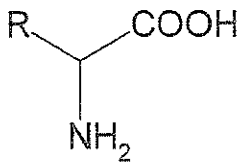
18. The transformant of any one of claims 15 to 17, wherein the enzyme having a coenzyme-regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

19. The transformant of any one of claims 14 to 18, which is obtained by transforming a host microorganism with the recombinant plasmid of claim 4 and a recombinant plasmid comprising a DNA encoding a polypeptide having an enzyme activity different from that of the D-amino acid oxidase as an insert.

20. A method of producing D-amino acid oxidase, comprising culturing a microorganism having an ability to produce the polypeptide of claim 1, accumulating the polypeptide in the culture, and harvesting the polypeptide.

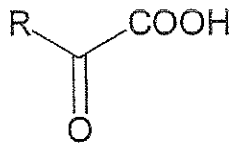
21. The production method of claim 20, wherein the microorganism is the transformant of any one of claims 12 to 19 or a microorganism belonging to the genus Candida.

22. A method of producing L-amino acids represented by the formula (2):



(2)

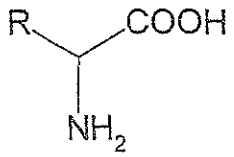
wherein R represents a C1-20 alkyl group optionally having substituent(s), a C7-20 aralkyl group optionally having substituent(s) or a C6-20 aryl group optionally having substituent(s), or 2-oxo acid represented by the formula (3):



(3)

wherein R represents as defined above, which comprises

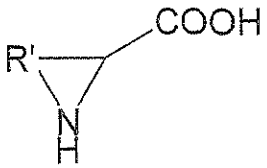
reacting the polypeptide of claim 1 or a microorganism having an ability to produce the polypeptide, with racemic amino acid represented by the formula (1):



(1)

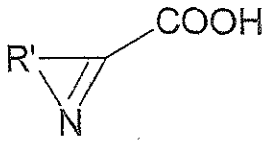
wherein R represents as defined above.

23. A method of producing cyclic L-amino acid represented by the formula (5):



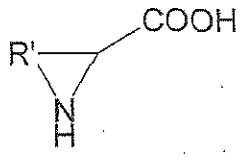
(5)

wherein R' is a C2-7 alkyl chain optionally having substituent(s), or cyclic imine represented by the formula (6):



(6)

wherein R' is as defined above, which comprises reacting the polypeptide of claim 1 or a microorganism having an ability to produce the polypeptide, with a cyclic amino acid represented by the formula (4):

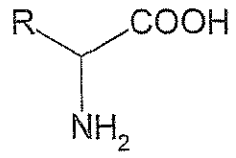


(4)

wherein R' is as defined above.

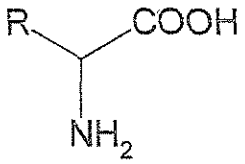
24. The production method of claim 22 or 23, in which the transformant of claim 12 or 13 is used.

25. A method of producing L-amino acids represented by the formula (2):



(2)

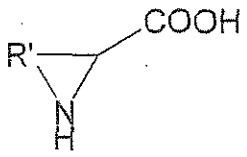
wherein R represents a C1-20 alkyl group optionally having substituent(s), a C7-20 aralkyl group optionally having substituent(s) or a C6-20 aryl group optionally having substituent(s), which comprises reacting, in the presence of an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity, the polypeptide of claim 1 or a microorganism having an ability to produce the polypeptide with racemic amino acid represented by the formula (1):



(1)

wherein R represents as defined above.

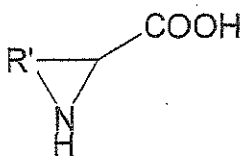
26. A method of producing cyclic L-amino acid represented by the formula (5):



(5)

wherein R' is a C2-7 alkyl chain optionally having substituent(s), which comprises reacting, in the presence of an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity, the polypeptide of claim 1 or a microorganism having an ability to produce the polypeptide with cyclic amino

acid represented by the formula (4):



(4)

wherein R′ is as defined above.

27. The production method of claim 25 or 26, wherein the amino acid dehydrogenase is a leucine dehydrogenase or a phenylalanine dehydrogenase.

28. The production method of any one of claims 25 to 27, wherein the enzyme having a coenzyme-regenerating activity is a formate dehydrogenase or a glucose dehydrogenase.

29. The production method of any one of claims 12 to 19, which comprises use of the transformant of any one of claims 25 to 28.

30. The production method of claim 29, which comprises use of a transformant coexpressing 3 enzymes of a D-amino acid oxidase, an amino acid dehydrogenase and an enzyme having a coenzyme-regenerating activity.