CLAIMS

1. An isolated polynucleotide encoding any one of the following amino acid sequences (A1) to (A3) or (A5):
(A1) an amino acid sequence represented SEQ ID NO: 3,
(A2) an amino acid sequence i) having at least 95% sequence identity to an amino acid sequence represented by SEQ ID NO: 3, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative,
(A3) an amino acid sequence represented by i) SEQ ID NO: 3 in which one or plural amino acids are deleted, substituted, or added, wherein the plural amino acids are 2, 3, 4, 5, 6, 7, or 10 amino acids, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative, or
(A5) an amino acid sequence i) represented by SEQ ID NO: 3 which is an altered amino acid sequence having one or more amino acid substitutions selected from the group consisting of substitution of the 109th valine by isoleucine, substitution of the 191st glycine by aspartic acid, and substitution of the 246th glutamine by arginine, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative.

2. The polynucleotide according to claim 1, which has an altered base sequence comprising a codon selected so that the frequency of use of codon of a base sequence encoding the amino acid sequence corresponds to the frequency of use of codon in E. coli.

3. A polynucleotide in which a promoter which can function in a host cell is connected with the polynucleotide according to claim 1 or 2 so that they can function.

4. A recombinant vector comprising the polynucleotide according to any one of claims 1 to 3.

5. A transformant in which the polynucleotide according to any one of claims 1 to 3 or the recombinant vector according to claim 4 is introduced into a host cell.

6. The transformant according to claim 5, wherein the host cell is a microorganism or E. coli.

7. A transformant having the polynucleotide according to any one of claims 1 to 3.

8. A method for producing a recombinant vector, which comprises the step of integrating the polynucleotide according to any one of claims 1 to 3 into a vector which can be replicated in a host cell.

9. A method for producing a transformant, which comprises the step of introducing the polynucleotide according to any one of claims 1 to 3 or the recombinant vector according to claim 4 into a host cell.

10. An isolated polynucleotide encoding any one of the

following amino acid sequences (B1) to (B3):
(B1) an amino acid sequence represented by SEQ ID NO: 1,
(B2) an amino acid sequence i) having at least 95% sequence identity to an amino acid sequence represented by SEQ ID NO: 1, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative,
(B3) an amino acid sequence represented by i) SEQ ID NO: 1 in which one or plural amino acids are deleted, substituted, or added, wherein the plural amino acids are 2, 3, 4, 5, 6, 7, 8, 10, 12, 15 or 17 amino acids, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative
.

11. A polynucleotide in which a promoter which can function in a host cell is connected with the polynucleotide according to claim 10 so that they can function.

12. A recombinant vector comprising the polynucleotide according to claim 10 or 11.

13. A transformant in which the polynucleotide according to claim 10 or 11 or the recombinant vector according to claim 12 is introduced into a host cell.

14. The transformant according to claim 13, wherein the host cell is a microorganism or E. coli.

15. A transformant having the polynucleotide according to claim 10 or 11.

16. A method for producing a recombinant vector, which comprises the step of integrating the polynucleotide according to claim 10 or 11 into a vector which can be replicated in a host cell.

17. A method for producing a transformant, which comprises the step of introducing the polynucleotide according to claim 10 or 11 or the recombinant vector according to claim 12 into a host cell.

18. A recombinant vector comprising the polynucleotide according to any one of claims 1 to 3 and the polynucleotide according to claim 10 or 11.

19. The recombinant vector according to claim 18, which further comprises a polynucleotide encoding an amino acid sequence of a protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound, or a polynucleotide in which the polynucleotide is connected with a promoter which can function in a host cell so that they can function.

20. The recombinant vector according to claim 19, wherein the protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound is a leucine dehydrogenase.

21. The recombinant vector according to claim 19 or 20, wherein the amino acid sequence of the protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound is any

one of the following amino acid sequences (C1) to (C3):
(C1) an amino acid sequence represented by SEQ ID NO: 7,
(C2) an amino acid sequence i) having at least 90% sequence identity to an amino acid sequence represented by SEQ ID NO: 7, and ii) of a protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound, or
(C3) an amino acid sequence i) represented by SEQ ID NO: 7 in which one or plural amino acids are deleted, substituted, or added, wherein the plural amino acids are 2, 3, 4, 5, 6, 7, 10, 15, 18, 20, 25, 30, 35, 36, or 40 amino acids, and ii) of a protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound.

22. A transformant in which the recombinant vector according to any one of claims 18 to 21 is introduced into a host cell.

23. The transformant according to claim 22, wherein the host cell is a microorganism or E. coli.

24. A transformant having the followings:
i) a polynucleotide having a base sequence encoding an amino acid sequence of a protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound, or a polynucleotide in which the polynucleotide is connected with a promoter which can function in a host cell so that they can function;
ii) the polynucleotide according to any one of claims 1 to 3; and
iii) the polynucleotide according to claim 10 or 11.

25. An isolated protein having any one of the following amino acid sequences (A1) to (A3) or (A5):
(A1) an amino acid sequence represented SEQ ID NO: 3,

(A2) an amino acid sequence i) having at least 95% sequence identity to an amino acid sequence represented by SEQ ID NO: 3, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative,
(A3) an amino acid sequence represented by i) SEQ ID NO: 3 in which one or plural amino acids are deleted, substituted, or added, wherein the plural amino acids are 2, 3, 4, 5, 6, 7, or 10 amino acids, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative, or
(A5) an amino acid sequence i) represented by SEQ ID NO: 3 which is an altered amino acid sequence having one or more amino acid substitutions selected from the group consisting of substitution of the 109th valine by isoleucine, substitution of the 191st glycine by aspartic acid, and substitution of the 246th glutamine by arginine, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative.

26. An isolated protein having any one of the following amino acid sequences (B1) to (B3):
(B1) an amino acid sequence represented by SEQ ID NO: 1,
(B2) an amino acid sequence i) having at least 95% sequence

identity to an amino acid sequence represented by SEQ ID NO: 1, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative, (B3) an amino acid sequence represented by i) SEQ ID NO: 1 in which one or plural amino acids are deleted, substituted, or added, wherein the plural amino acids are 2, 3, 4, 5, 6, 7, 8, 10, 12, 15 or 17 amino acids, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative, or

.

27. A method for producing an L-α-amino acid compound, which comprises (1) the step of reacting any one or both of a protein having any one of the following amino acid sequences (A1) to (A3) or (A5):
(A1) an amino acid sequence represented SEQ ID NO: 3,
(A2) an amino acid sequence i) having at least 95% sequence identity to an amino acid sequence represented by SEQ ID NO: 3, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative,
(A3) an amino acid sequence represented by i) SEQ ID NO: 3 in which one or plural amino acids are deleted, substituted, or added, wherein the plural amino acids are 2, 3, 4, 5, 6, 7, or

10 amino acids, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative, or

(A5) an amino acid sequence i) represented by SEQ ID NO: 3 which is an altered amino acid sequence having one or more amino acid substitutions selected from the group consisting of substitution of the 109th valine by isoleucine, substitution of the 191st glycine by aspartic acid, and substitution of the 246th glutamine by arginine, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative;

and a protein having any one of the following amino acid sequences (B1) to (B3):

(B1) an amino acid sequence represented by SEQ ID NO: 1,

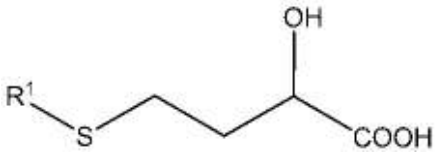
(B2) an amino acid sequence i) having at least 95% sequence identity to an amino acid sequence represented by SEQ ID NO: 1, and ii) of a protein having any one or both of the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative, or

(B3) an amino acid sequence represented by i) SEQ ID NO: 1 in which one or plural amino acids are deleted, substituted, or added, wherein the plural amino acids are 2, 3, 4, 5, 6, 7, 8, 10, 12, 15 or 17 amino acids, and ii) of a protein having any one or both of the ability to oxidize a

2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative and the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative,
with an α-hydroxycarboxylic acid compound to obtain a corresponding α-oxocarboxylic acid compound, and
(2) the step of reacting a protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound with the α-oxocarboxylic acid compound obtained in the step (1) to obtain a corresponding L-α-amino acid compound.

28. The production method according to claim 27, wherein the α-hydroxycarboxylic acid compound is a sulfur-containing α-hydroxycarboxylic acid compound, the corresponding α-oxocarboxylic acid compound is a sulfur-containing α-oxocarboxylic acid compound, the corresponding L-α-amino acid compound is a sulfur-containing L-α-amino acid compound, the protein reacted with an α-hydroxycarboxylic acid compound in the step (1) is a protein at least having the ability to oxidize a 2-hydroxy-4-(methylthio)butyric acid derivative and convert the same into a corresponding 2-oxo-4-(methylthio)butyric acid derivative.

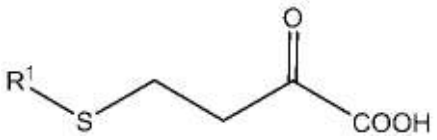
29. The production method according to claim 28, wherein the sulfur-containing α-hydroxycarboxylic acid compound is a compound represented by formula (1):



(1)

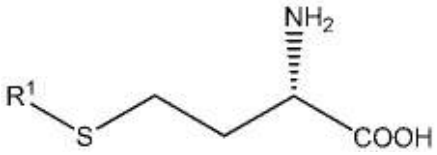
wherein R^1 represents a hydrogen atom or an optionally substituted C1-8 alkyl group;
the sulfur-containing α-oxocarboxylic acid compound is a

compound represented by formula (2):



(2)

wherein R^1 is the same as defined above;
and the sulfur-containing L-α-amino acid compound is a compound represented by formula (3):



(3)

wherein R^1 is the same as defined above.

30. The production method according to claim 27, wherein the α-hydroxycarboxylic acid compound is α-hydroxy-isocaproic acid, the corresponding α-oxocarboxylic acid compound is α-oxo-isocaproic acid, the corresponding L-α-amino acid compound is L-leucine, and the protein reacted with an α-hydroxycarboxylic acid compound in the step (1) is a protein at least having the ability to oxidize an α-hydroxy-isocaproic acid derivative and convert the same into a corresponding α-oxo-isocaproic acid derivative.

31. The production method according to any one of claims 27 to 30, wherein the protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound is a leucine dehydrogenase.

32. The production method according to any one of claims 27 to 31, wherein the amino acid sequence of a protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound is any one of the following amino acid sequences (C1) to (C3):
(C1) an amino acid sequence represented by SEQ ID NO: 7,

(C2) an amino acid sequence i) having at least 90% sequence identity to an amino acid sequence represented by SEQ ID NO: 7, and ii) of a protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound, or
(C3) an amino acid sequence i) represented by SEQ ID NO: 7 in which one or plural amino acids are deleted, substituted, or added, wherein the plural amino acids are 2, 3, 4, 5, 6, 7, 10, 15, 18, 20, 25, 30, 35, 36, or 40 amino acids, and ii) of a protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound.

33. The production method according to any one of claims 27 to 32, wherein the protein reacted with an α-hydroxycarboxylic acid compound in the step (1) is provided in a reaction system in the form in which the protein is included in a transformant in which a polynucleotide encoding the protein is introduced into a host cell or in a treated product thereof.

34. The production method according to claim 33, wherein the transformant is the transformant according to any one of claims 5, 6, 7, 13, 14, 15, 22, 23, and 24.

35. The production method according to any one of claims 27 to 34, wherein the protein having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound is provided in a reaction system in the form in which the protein is included in a transformant in which a polynucleotide encoding the protein is introduced into a host cell or in a treated product thereof.

36. The production method according to claim 35, wherein the transformant is the transformant according to any one of claims 22, 23, and 24.

37. The production method according to any one of claims 27 to 36, wherein the step (1) is performed in the presence of a protein having the ability to convert a reduced β-nicotinamide adenine dinucleotide or a reduced β-nicotinamide adenine dinucleotide phosphate into its oxidized form.

38. The production method according to claim 37, wherein the protein having the ability to convert a reduced β-nicotinamide adenine dinucleotide or a reduced β-nicotinamide adenine dinucleotide phosphate into its oxidized form is a protein further having the ability to aminate an α-oxocarboxylic acid compound and convert the same into a corresponding L-α-amino acid compound.

39. The production method according to claim 37 or 38, wherein the protein having the ability to convert a reduced β-nicotinamide adenine dinucleotide or a reduced β-nicotinamide adenine dinucleotide phosphate into its oxidized form is provided in a reaction system in the form in which the protein is included in a transformant in which a polynucleotide encoding the protein is introduced into a host cell or in a treated product thereof.

40. The production method according to any one of claims 27 to 39, wherein the step (2) is performed in the presence of a protein having the ability to convert an oxidized β-nicotinamide adenine dinucleotide or an oxidized β-nicotinamide adenine dinucleotide phosphate into its reduced form.

41. The production method according to claim 40, wherein the protein having the ability to convert an oxidized β-nicotinamide adenine dinucleotide or an oxidized β-nicotinamide adenine dinucleotide phosphate into its reduced

form is a protein further having the ability to oxidize an α-hydroxycarboxylic acid compound and convert the same into a corresponding α-oxocarboxylic acid compound.

42. The production method according to claim 40 or 41, wherein the protein having the ability to convert an oxidized β-nicotinamide adenine dinucleotide or an oxidized β-nicotinamide adenine dinucleotide phosphate into its reduced form is provided in a reaction system in the form in which the protein is included in a transformant in which a polynucleotide encoding the protein is introduced into a host cell or in a treated product thereof.

43. The production method according to any one of claims 27 to 42, wherein the step (1) and the step (2) are performed in one reaction system.