Therefore, conditions required for xylanase vary widely. Examples thereof include severe conditions in which enzymes easily inactivate, such as a condition involving a pH of from 4 to 10, a temperature of from 40 °C to 80 °C, and a usage time of several days.

In order to address these various needs, many types of mutant xylanases and novel xylanases have been reported. However, xylanases that can work with sufficient stability under severe conditions in which enzymes easily inactivate have not been found.

[0022] Mutant xylanases obtained in order to improve heat resistance have a problem in that the initial rate of reaction largely decreases. It is presumed that the reason therefor is a decrease in the structural flexibility of the entire protein caused by mutations or the like of the amino acid sequence that has been introduced in order to improve heat resistance.

[0023] In such circumstances, development of a xylanase which exhibits stable activity for a predetermined period of time under severe conditions in which enzymes easily inactivate such as an acidic region (from pH 4 to 6), a basic region (from pH 8 to 10), or a high temperature range (from 40 °C to 80 °C), and with which the initial rate of reaction is not significantly reduced as compared to a wild-type xylanase corresponding thereto.

[0024] The present invention aims to provide an inexpensive and efficient saccharification method for lignocellulose using a thermostable xylanase. The invention also aims to provide a mutant xylanase that has a substitute amino acid residue, and that exhibits stable activity even under severe conditions in which enzymes easily inactivate, and that provides an initial rate of reaction not significantly reduced as compared to a wild-type xylanase corresponding to the mutant xylanase. The invention also aims to provide a production method capable of producing the mutant xylanase at low cost, as well as provide various uses of the mutant xylanase.

Means for Solving Problem

In an aspect, there is provided a method of producing a saccharified product of lignocellulose, the method comprising contacting a lignocellulosic raw material with a mutant xylanase comprising at least the following substitute amino acid residues in an amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing: a leucine residue substituted for an asparagine residue at position 29; an arginine residue substituted for a lysine residue at position 58; an amino acid residue, other than a tyrosine residue, substituted for a tyrosine residue at position 27; and a serine residue substituted for an asparagine residue at position 44.

In another aspect, there is provided a method of producing a saccharified product of lignocellulose, the method comprising contacting a lignocellulosic raw material with a mutant xylanase comprising at least substitution of an amino acid residue at position 154 in the amino

acid sequence represented by SEQ ID NO: 2 in the Sequence Listing with a methionine residue, the mutant xylanase providing an initial rate of reaction that is at least 70% of that provided by a wild-type xylanase corresponding thereto, that has a xylanase activity after heat treatment at 50 °C for 24 hours that is at least 50% of its xylanase activity before the heat treatment, and that has a substitute amino acid residue, wherein the initial rate of reaction is an initial rate of reaction of xylanase activity as determined by: vigorously mixing 1% (w/w) birchwood xylan as a substrate into 100 mM a sodium citrate buffer solution (pH 4.5); performing centrifugation at 5000×g for 15 minutes to prepare a supernatant from which residual xylan present in the sodium citrate buffer solution has been removed; mixing a xylanase into the supernatant as a substrate solution such that the amount of the xylanase is 0.1% (w/w) with respect to the substrate solution; allowing the resultant mixture to react while being stirred at 45 °C for 30 minutes; and measuring an amount of reducing saccharide in the obtained reaction solution by the DNS method to obtain the initial rate of reaction of the xylanase activity can be obtained.

In aspects, there are provided methods of producing a saccharified product, the method comprising: recovering the thermostable xylanase from a saccharification reaction solution containing the saccharified product of lignocellulose obtained by the method of producing a saccharified product as defined above; and contacting the recovered thermostable xylanase with a lignocellulosic raw material, to produce a saccharified product.

In another aspect, there is provided a mutant xylanase comprising at least the following substitute amino acid residues in an amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing: a leucine residue substituted for an asparagine residue at position 29; an arginine residue substituted for a lysine residue at position 58; an amino acid residue, other than a tyrosine residue, substituted for a tyrosine residue at position 27; and a serine residue substituted for an asparagine residue at position 44.

In another aspect, there is provided a mutant xylanase comprising at least substitution of a leucine residue at position 154 with a methionine residue in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing, the mutant xylanase providing an initial rate of reaction that is at least 70% of that provided by a wild-type xylanase corresponding thereto, that has a xylanase activity after heat treatment at 50 °C for 24 hours that is at least 50% of its xylanase activity before the heat treatment, and that has a substitute amino acid residue, wherein the initial rate of reaction is an initial rate of reaction of xylanase activity as determined by: vigorously mixing 1% (w/w) birchwood xylan as a substrate into 100 mM a sodium citrate buffer solution (pH 4.5); performing centrifugation at 5000×g for 15 minutes to prepare a supernatant from which residual xylan present in the sodium citrate

buffer solution has been removed; mixing a xylanase into the supernatant as a substrate solution such that the amount of the xylanase is 0.1% (w/w) with respect to the substrate solution; allowing the resultant mixture to react while being stirred at 45 °C for 30 minutes; and measuring an amount of reducing saccharide in the obtained reaction solution by the DNS method to obtain the initial rate of reaction of the xylanase activity can be obtained.

In another aspect, there is provided a mutant xylanase comprising at least the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing: an aspartic acid residue substituted for an asparagine residue at position 33; an arginine residue substituted for a glycine residue at position 36; a serine residue substituted for a threonine residue at position 90; an arginine residue substituted for a glutamine residue at position 132; a methionine residue substituted for the leucine residue at position 154; a threonine residue substituted for a serine residue at position 174; a histidine residue substituted for a proline residue at position 195; an asparagine residue substituted for a serine residue at position 197; and a glutamic acid residue substituted for a glycine residue at position 217.

In another aspect, there is provided a mutant xylanase comprising at least the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing: a valine residue substituted for an isoleucine residue at position 30; an aspartic acid residue substituted for an asparagine residue at position 33; an arginine residue substituted for a glycine residue at position 36; and a methionine residue substituted for the leucine residue at position 154.

In another aspect, there is provided a mutant xylanase comprising at least the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing: a valine residue substituted for an isoleucine residue at position 30; a threonine residue substituted for a serine residue at position 59; a methionine residue substituted for the leucine residue at position 154; a histidine residue substituted for a tyrosine residue at position 239; and a serine residue substituted for a cysteine residue at position 242.

In another aspect, there is provided a mutant xylanase having a homology of 95% or higher with any of the following amino acid sequences:

an amino acid sequence comprising only the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2:

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36;

a serine residue substituted for a threonine residue at position 90;

an arginine residue substituted for a glutamine residue at position 132;

a methionine residue substituted for a leucine residue at position 154;

a threonine residue substituted for a serine residue at position 174;

a histidine residue substituted for a proline residue at position 195;

an asparagine residue substituted for a serine residue at position 197; and

a glutamic acid residue substituted for a glycine residue at position 217,

an amino acid sequence comprising only the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2:

a valine residue substituted for an isoleucine residue at position 30;

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36; and

a methionine residue substituted for a leucine residue at position 154, or

an amino acid sequence comprising only the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2:

a valine residue substituted for an isoleucine residue at position 30;

a threonine residue substituted for a serine residue at position 59;

a methionine residue substituted for a leucine residue at position 154;

a histidine residue substituted for a tyrosine residue at position 239; and

a serine residue substituted for a cysteine residue at position 242.

In another aspect, there is provided a nucleic acid represented by a base sequence encoding the amino acid sequence of the mutant xylanase as defined above.

In another aspect, there is provided an expression vector comprising the nucleic acid as defined above.

In another aspect, there is provided a transformant comprising the expression vector as defined above.

In another aspect, there is provided a method of producing a mutant xylanase, the method comprising culturing the transformant as defined above and recovering the mutant xylanase as defined above from at least one of the cultured transformant or a culture product of the transformant.

In another aspect, there is provided a mutant xylanase produced by the production method as defined above.

In another aspect, there is provided a composition comprising the mutant xylanase as defined above.

In another aspect, there is provided a method of bleaching a pulp, the method comprising contacting the mutant xylanase as defined above with the pulp.

In another aspect, there is provided a detergent comprising the mutant xylanase as defined above.

In another aspect, there is provided an animal feed comprising the mutant xylanase as defined above.

In another aspect, there is provided a bread-making modifier comprising the mutant xylanase as defined above.

[0025] The present ~~invention~~ disclosure includes the following:

[1] A method of producing a saccharified product of lignocellulose, the method including contacting a lignocellulosic raw material with a thermostable xylanase.

[2] The method of producing a saccharified product according to [1], wherein the lignocellulosic raw material is pulp.

[3] A method of producing a saccharified product, the method including:

recovering the thermostable xylanase from a saccharification reaction solution containing the saccharified product of lignocellulose obtained by the method of producing a saccharified product according to [1] or [2]; and

contacting the recovered thermostable xylanase with a lignocellulosic raw material, to produce a saccharified product.

CLAIMS

1. A method of producing a saccharified product of lignocellulose, the method comprising contacting a lignocellulosic raw material with a mutant xylanase comprising at least the following substitute amino acid residues in an amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing:

a leucine residue substituted for an asparagine residue at position 29;

an arginine residue substituted for a lysine residue at position 58;

an amino acid residue, other than a tyrosine residue, substituted for a tyrosine residue at position 27; and

a serine residue substituted for an asparagine residue at position 44.

2. The method of producing a saccharified product according to claim 1, wherein the lignocellulosic raw material is pulp.

3. A method of producing a saccharified product, the method comprising:

recovering the thermostable xylanase from a saccharification reaction solution containing the saccharified product of lignocellulose obtained by the method of producing a saccharified product according to claim 1 or claim 2; and

contacting the recovered thermostable xylanase with a lignocellulosic raw material, to produce a saccharified product.

4. The method of producing a saccharified product according to claim 3, wherein the saccharification reaction solution is subjected to solid-liquid separation using centrifugation or a microfiltration membrane, and the separated liquid is ultrafiltered using an ultrafiltration membrane to separate and recover the saccharified product of lignocellulose and the thermostable xylanase.

5. The method of producing a saccharified product according to claim 4, wherein the method comprises contacting a solid obtained by the solid-liquid separation using centrifugation or a microfiltration membrane and the thermostable xylanase recovered using the ultrafiltration membrane with a lignocellulosic raw material, to produce a saccharified product.

6. The method of producing a saccharified product according to any one of claims 1 to 5, wherein the thermostable xylanase is a mutant xylanase that provides an initial rate of reaction that is at least 70% of that provided by a wild-type xylanase corresponding thereto, that has a xylanase activity after heat treatment at 50 °C for 24 hours that is at least 50% of its xylanase activity before the heat treatment, and that has a substitute amino acid residue,

wherein the initial rate of reaction is an initial rate of reaction of xylanase activity as determined by:

vigorously mixing 1% (w/w) birchwood xylan as a substrate into 100 mM a sodium citrate buffer solution (pH 4.5);

performing centrifugation at 5000×g for 15 minutes to prepare a supernatant from which residual xylan present in the sodium citrate buffer solution has been removed;

mixing a xylanase into the supernatant as a substrate solution such that the amount of the xylanase is 0.1% (w/w) with respect to the substrate solution;

allowing the resultant mixture to react while being stirred at 45 °C for 30 minutes; and

measuring an amount of reducing saccharide in the obtained reaction solution by the DNS method to obtain the initial rate of reaction of the xylanase activity can be obtained.

7. The method of producing a saccharified product according to any one of claims 1 to 6, wherein, in the mutant xylanase used in the producing of a saccharified product, the amino acid residue, other than a tyrosine residue, substituted for the tyrosine residue at position 27 is a phenylalanine residue.

8. A method of producing a saccharified product of lignocellulose, the method comprising contacting a lignocellulosic raw material with a mutant xylanase comprising at least substitution of an amino acid residue at position 154 in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing with a methionine residue, the mutant xylanase providing an initial rate of reaction that is at least 70% of that provided by a wild-type xylanase corresponding thereto, that has a xylanase activity after heat treatment at 50 °C for 24 hours that is at least 50% of its xylanase activity before the heat treatment, and that has a substitute amino acid residue,

wherein the initial rate of reaction is an initial rate of reaction of xylanase activity as determined by:

vigorously mixing 1% (w/w) birchwood xylan as a substrate into 100 mM a sodium

citrate buffer solution (pH 4.5);

performing centrifugation at 5000×g for 15 minutes to prepare a supernatant from which residual xylan present in the sodium citrate buffer solution has been removed;

mixing a xylanase into the supernatant as a substrate solution such that the amount of the xylanase is 0.1% (w/w) with respect to the substrate solution;

allowing the resultant mixture to react while being stirred at 45 °C for 30 minutes; and

measuring an amount of reducing saccharide in the obtained reaction solution by the DNS method to obtain the initial rate of reaction of the xylanase activity can be obtained.

9. The method of producing a saccharified product according to claim 8, wherein the mutant xylanase used in the producing of a saccharified product includes at least the following substitute amino acid residues:

an aspartic acid residue substituted for an asparagine residue at position 33;
an arginine residue substituted for a glycine residue at position 36;
a serine residue substituted for a threonine residue at position 90;
an arginine residue substituted for a glutamine residue at position 132;
a methionine residue substituted for a leucine residue at position 154;
a threonine residue substituted for a serine residue at position 174;
a histidine residue substituted for a proline residue at position 195;
an asparagine residue substituted for a serine residue at position 197; and
a glutamic acid residue substituted for a glycine residue at position 217.

10. The method of producing a saccharified product according to claim 8, wherein the mutant xylanase used in the producing of a saccharified product includes at least the following substitute amino acid residues:

a valine residue substituted for an isoleucine residue at position 30;
an aspartic acid residue substituted for an asparagine residue at position 33;
an arginine residue substituted for a glycine residue at position 36; and
a methionine residue substituted for a leucine residue at position 154.

11. The method of producing a saccharified product according to claim 8, wherein the mutant xylanase used in the producing of a saccharified product includes at least the following substitute amino acid residues:

a valine residue substituted for an isoleucine residue at position 30;
a threonine residue substituted for a serine residue at position 59;
a methionine residue substituted for a leucine residue at position 154;
a histidine residue substituted for a tyrosine residue at position 239; and
a serine residue substituted for a cysteine residue at position 242.

12. The method of producing a saccharified product according to claim 8, wherein the mutant xylanase has a homology of 95% or higher with any of the following amino acid sequences:

an amino acid sequence comprising only the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2:

an aspartic acid residue substituted for an asparagine residue at position 33;
an arginine residue substituted for a glycine residue at position 36;
a serine residue substituted for a threonine residue at position 90;
an arginine residue substituted for a glutamine residue at position 132;
a methionine residue substituted for a leucine residue at position 154;
a threonine residue substituted for a serine residue at position 174;
a histidine residue substituted for a proline residue at position 195;
an asparagine residue substituted for a serine residue at position 197; and
a glutamic acid residue substituted for a glycine residue at position 217,

an amino acid sequence comprising only the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2:

a valine residue substituted for an isoleucine residue at position 30;
an aspartic acid residue substituted for an asparagine residue at position 33;
an arginine residue substituted for a glycine residue at position 36; and
a methionine residue substituted for a leucine residue at position 154, or

an amino acid sequence comprising only the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2:

a valine residue substituted for an isoleucine residue at position 30;
a threonine residue substituted for a serine residue at position 59;
a methionine residue substituted for a leucine residue at position 154;
a histidine residue substituted for a tyrosine residue at position 239; and
a serine residue substituted for a cysteine residue at position 242.

13. The method of producing a saccharified product according to any one of claims 8 to 12, wherein the lignocellulosic raw material is pulp.

14. A method of producing a saccharified product, the method comprising:

recovering the thermostable xylanase from a saccharification reaction solution containing the saccharified product of lignocellulose obtained by the method of producing a saccharified product according to any one of claims 8 to 13; and

contacting the recovered thermostable xylanase with a lignocellulosic raw material, to produce a saccharified product.

15. The method of producing a saccharified product according to claim 14, wherein the saccharification reaction solution is subjected to solid-liquid separation using centrifugation or a microfiltration membrane, and the separated liquid is ultrafiltered using an ultrafiltration membrane to separate and recover the saccharified product of lignocellulose and the thermostable xylanase.

16. The method of producing a saccharified product according to claim 15, wherein the method comprises contacting a solid obtained by the solid-liquid separation using centrifugation or a microfiltration membrane and the thermostable xylanase recovered using the ultrafiltration membrane with a lignocellulosic raw material, to produce a saccharified product.

17. A mutant xylanase comprising at least the following substitute amino acid residues in an amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing:

a leucine residue substituted for an asparagine residue at position 29;

an arginine residue substituted for a lysine residue at position 58;

an amino acid residue, other than a tyrosine residue, substituted for a tyrosine residue at position 27; and

a serine residue substituted for an asparagine residue at position 44.

18. The mutant xylanase according to claim 17, wherein the amino acid residue, other than a tyrosine residue, substituted for the tyrosine residue at position 27 in the amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing is a phenylalanine residue.

19. A mutant xylanase comprising at least substitution of a leucine residue at position 154 with a methionine residue in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing,

the mutant xylanase providing an initial rate of reaction that is at least 70% of that provided by a wild-type xylanase corresponding thereto, that has a xylanase activity after heat treatment at 50 °C for 24 hours that is at least 50% of its xylanase activity before the heat treatment, and that has a substitute amino acid residue,

wherein the initial rate of reaction is an initial rate of reaction of xylanase activity as determined by:

vigorously mixing 1% (w/w) birchwood xylan as a substrate into 100 mM a sodium citrate buffer solution (pH 4.5);

performing centrifugation at 5000×g for 15 minutes to prepare a supernatant from which residual xylan present in the sodium citrate buffer solution has been removed;

mixing a xylanase into the supernatant as a substrate solution such that the amount of the xylanase is 0.1% (w/w) with respect to the substrate solution;

allowing the resultant mixture to react while being stirred at 45 °C for 30 minutes; and

measuring an amount of reducing saccharide in the obtained reaction solution by the DNS method to obtain the initial rate of reaction of the xylanase activity can be obtained.

20. A mutant xylanase comprising at least the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing:

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36;

a serine residue substituted for a threonine residue at position 90;

an arginine residue substituted for a glutamine residue at position 132;

a methionine residue substituted for the leucine residue at position 154;

a threonine residue substituted for a serine residue at position 174;

a histidine residue substituted for a proline residue at position 195;

an asparagine residue substituted for a serine residue at position 197; and

a glutamic acid residue substituted for a glycine residue at position 217.

21. A mutant xylanase comprising at least the following substitute amino acid

residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing:

a valine residue substituted for an isoleucine residue at position 30;

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36; and

a methionine residue substituted for the leucine residue at position 154.

22. A mutant xylanase comprising at least the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing:

a valine residue substituted for an isoleucine residue at position 30;

a threonine residue substituted for a serine residue at position 59;

a methionine residue substituted for the leucine residue at position 154;

a histidine residue substituted for a tyrosine residue at position 239; and

a serine residue substituted for a cysteine residue at position 242.

23. A mutant xylanase having a homology of 95% or higher with any of the following amino acid sequences:

an amino acid sequence comprising only the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2:

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36;

a serine residue substituted for a threonine residue at position 90;

an arginine residue substituted for a glutamine residue at position 132;

a methionine residue substituted for a leucine residue at position 154;

a threonine residue substituted for a serine residue at position 174;

a histidine residue substituted for a proline residue at position 195;

an asparagine residue substituted for a serine residue at position 197; and

a glutamic acid residue substituted for a glycine residue at position 217,

an amino acid sequence comprising only the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2:

a valine residue substituted for an isoleucine residue at position 30;

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36; and

a methionine residue substituted for a leucine residue at position 154, or

an amino acid sequence comprising only the following substitute amino acid residues

in the amino acid sequence represented by SEQ ID NO: 2:

a valine residue substituted for an isoleucine residue at position 30;
a threonine residue substituted for a serine residue at position 59;
a methionine residue substituted for a leucine residue at position 154;
a histidine residue substituted for a tyrosine residue at position 239; and
a serine residue substituted for a cysteine residue at position 242.

24. A nucleic acid represented by a base sequence encoding the amino acid sequence of the mutant xylanase according to any one of claims 17 to 23.

25. An expression vector comprising the nucleic acid according to claim 24.

26. A transformant comprising the expression vector according to claim 25.

27. The transformant according to claim 26, wherein a host cell of the transformant is a cell derived from *Escherichia coli*, *Bacillus subtilis*, yeast, an actinomycete, or a filamentous fungus.

28. The transformant according to claim 27, wherein the filamentous fungus belongs to the genus *Trichoderma*, the genus *Acremonium*, the genus *Humicola*, or the genus *Aspergillus*.

29. The transformant according to claim 27 or claim 28, wherein the filamentous fungus is *Trichoderma viride*, *Acremonium cellulolyticus*, *Humicola insolens*, or *Aspergillus niger*.

30. A method of producing a mutant xylanase, the method comprising culturing the transformant according to any one of claims 26 to 29 and recovering the mutant xylanase according to any one of claims 17 to 23 from at least one of the cultured transformant or a culture product of the transformant.

31. A mutant xylanase produced by the production method according to claim 30.

32. A composition comprising the mutant xylanase according to any one of claims

17 to 23 and 31.

33. A method of bleaching a pulp, the method comprising contacting the mutant xylanase according to any one of claims 17 to 23 and 31 with the pulp.

34. A detergent comprising the mutant xylanase according to any one of claims 17 to 23 and 31.

35. An animal feed comprising the mutant xylanase according to any one of claims 17 to 23 and 31.

36. A bread-making modifier comprising the mutant xylanase according to any one of claims 17 to 23 and 31.

CLAIMS

1. A method of producing a saccharified product of lignocellulose, the method comprising contacting a lignocellulosic raw material with a thermostable xylanase.

2. The method of producing a saccharified product according to claim 1, wherein the lignocellulosic raw material is pulp.

3. A method of producing a saccharified product, the method comprising:

recovering the thermostable xylanase from a saccharification reaction solution containing the saccharified product of lignocellulose obtained by the method of producing a saccharified product according to claim 1 or claim 2; and

contacting the recovered thermostable xylanase with a lignocellulosic raw material, to produce a saccharified product.

4. The method of producing a saccharified product according to claim 3, wherein the saccharification reaction solution is subjected to solid-liquid separation using centrifugation or a microfiltration membrane, and the separated liquid is ultrafiltered using an ultrafiltration membrane to separate and recover the saccharified product of lignocellulose and the thermostable xylanase.

5. The method of producing a saccharified product according to claim 4, wherein the method comprises contacting a solid obtained by the solid-liquid separation using centrifugation or a microfiltration membrane and the thermostable xylanase recovered using the ultrafiltration membrane with a lignocellulosic raw material, to produce a saccharified product.

6. The method of producing a saccharified product according to any one of claims 1 to 5, wherein the thermostable xylanase is a mutant xylanase that provides an initial rate of reaction that is at least 70% of that provided by a wild-type xylanase corresponding thereto, that has a xylanase activity after heat treatment at 50 °C for 24 hours that is at least 50% of its xylanase activity before the heat treatment, and that has a substitute amino acid residue.

7. The method of producing a saccharified product according to claim 6, wherein

the mutant xylanase is a mutant xylanase comprising at least the following substitute amino acid residues in an amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing:

a leucine residue substituted for an asparagine residue at position 29;

an arginine residue substituted for a lysine residue at position 58;

an amino acid residue, other than a tyrosine residue, substituted for a tyrosine residue at position 27; and

an amino acid residue, other than an asparagine residue, substituted for an asparagine residue at position 44.

8. The method of producing a saccharified product according to claim 7, wherein, in the mutant xylanase used in the producing of a saccharified product, the amino acid residue, other than a tyrosine residue, substituted for the tyrosine residue at position 27 is a phenylalanine residue, and the amino acid residue, other than an asparagine residue, substituted for an asparagine residue at position 44 is a serine residue.

9. The method of producing a saccharified product according to claim 6, wherein the mutant xylanase is a mutant xylanase in which at least an amino acid residue at position 154 in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing is substituted with another amino acid residue.

10. The method of producing a saccharified product according to claim 9, wherein the mutant xylanase used in the producing of a saccharified product includes at least the following substitute amino acid residues:

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36;

a serine residue substituted for a threonine residue at position 90;

an arginine residue substituted for a glutamine residue at position 132;

a methionine residue substituted for a leucine residue at position 154;

a threonine residue substituted for a serine residue at position 174;

a histidine residue substituted for a proline residue at position 195;

an asparagine residue substituted for a serine residue at position 197; and

a glutamic acid residue substituted for a glycine residue at position 217.

11. The method of producing a saccharified product according to claim 9, wherein

the mutant xylanase used in the producing of a saccharified product includes at least the following substitute amino acid residues:

a valine residue substituted for an isoleucine residue at position 30;

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36; and

a methionine residue substituted for a leucine residue at position 154.

12. The method of producing a saccharified product according to claim 9, wherein the mutant xylanase used in the producing of a saccharified product includes at least the following substitute amino acid residues:

a valine residue substituted for an isoleucine residue at position 30;

a threonine residue substituted for a serine residue at position 59;

a methionine residue substituted for a leucine residue at position 154;

a histidine residue substituted for a tyrosine residue at position 239; and

a serine residue substituted for a cysteine residue at position 242.

13. A mutant xylanase comprising at least the following substitute amino acid residues in an amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing:

a leucine residue substituted for an asparagine residue at position 29;

an arginine residue substituted for a lysine residue at position 58;

an amino acid residue, other than a tyrosine residue, substituted for a tyrosine residue at position 27; and

an amino acid residue, other than an asparagine residue, substituted for an asparagine residue at position 44.

14. The mutant xylanase according to claim 13, wherein the amino acid residue, other than a tyrosine residue, substituted for the tyrosine residue at position 27 in the amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing is a phenylalanine residue, and the amino acid residue, other than an asparagine residue, substituted for an asparagine residue at position 44 in the amino acid sequence represented by SEQ ID NO: 1 in the Sequence Listing is a serine residue.

15. A mutant xylanase comprising substitution of at least a leucine residue at position 154 with another amino acid residue in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing.

16. The mutant xylanase according to claim 15, wherein the mutant xylanase comprises at least the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing:

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36;

a serine residue substituted for a threonine residue at position 90;

an arginine residue substituted for a glutamine residue at position 132;

a methionine residue substituted for the leucine residue at position 154;

a threonine residue substituted for a serine residue at position 174;

a histidine residue substituted for a proline residue at position 195;

an asparagine residue substituted for a serine residue at position 197; and

a glutamic acid residue substituted for a glycine residue at position 217.

17. The mutant xylanase according to claim 15, wherein the mutant xylanase comprises at least the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing:

a valine residue substituted for an isoleucine residue at position 30;

an aspartic acid residue substituted for an asparagine residue at position 33;

an arginine residue substituted for a glycine residue at position 36; and

a methionine residue substituted for the leucine residue at position 154.

18. The mutant xylanase according to claim 15, wherein the mutant xylanase comprises at least the following substitute amino acid residues in the amino acid sequence represented by SEQ ID NO: 2 in the Sequence Listing:

a valine residue substituted for an isoleucine residue at position 30;

a threonine residue substituted for a serine residue at position 59;

a methionine residue substituted for the leucine residue at position 154;

a histidine residue substituted for a tyrosine residue at position 239; and

a serine residue substituted for a cysteine residue at position 242.

19. A nucleic acid represented by a base sequence encoding the amino acid sequence of the mutant xylanase according to any one of claims 13 to 18.

20. An expression vector comprising the nucleic acid according to claim 19.

21. A transformant comprising the expression vector according to claim 20.

22. The transformant according to claim 21, wherein a host cell of the transformant is a cell derived from *Escherichia coli*, *Bacillus subtilis*, yeast, an actinomycete, or a filamentous fungus.

23. The transformant according to claim 22, wherein the filamentous fungus belongs to the genus *Trichoderma*, the genus *Acremonium*, the genus *Humicola*, or the genus *Aspergillus*.

24. The transformant according to claim 22 or claim 23, wherein the filamentous fungus is *Trichoderma viride*, *Acremonium cellulolyticus*, *Humicola insolens*, or *Aspergillus niger*.

25. A method of producing a mutant xylanase, the method comprising culturing the transformant according to any one of claims 21 to 24 and recovering the mutant xylanase according to any one of claims 13 to 18 from at least one of the cultured transformant or a culture product of the transformant.

26. A mutant xylanase produced by the production method according to claim 25.

27. A composition comprising the mutant xylanase according to any one of claims 13 to 18 and 21.

28. A method of bleaching a pulp, the method comprising contacting the mutant xylanase according to any one of claims 13 to 18 and 21 with the pulp.

29. A detergent comprising the mutant xylanase according to any one of claims 13 to 18 and 21.

30. An animal feed comprising the mutant xylanase according to any one of claims 13 to 18 and 21.

31. A bread-making modifier comprising the mutant xylanase according to any one

of claims 13 to 18 and 21.