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Cheng et al.

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(54) **XYLOSIDASE HAVING IMPROVED ENZYMATIC ACTIVITY**

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C12N 15/52	(2006.01)
C12N 15/10	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/52*** (2013.01); ***C12N 9/2402*** (2013.01); ***C12N 15/102*** (2013.01); ***C12Y 302/01037*** (2013.01); *C12Y 302/01072* (2013.01); *C12Y 302/01177* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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Primary Examiner — Richard C Ekstrom

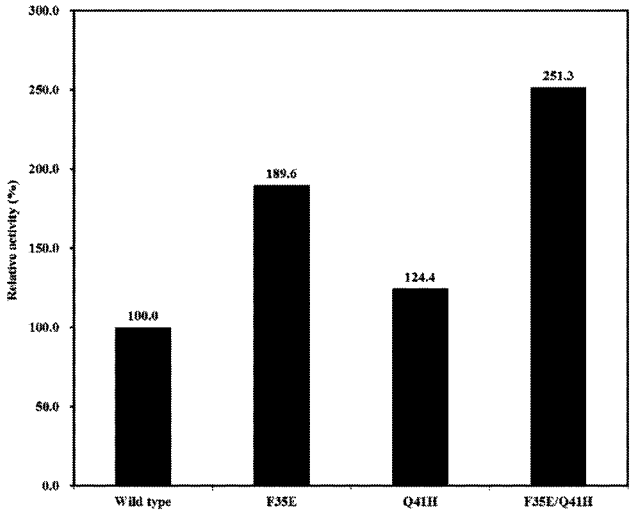
(74) *Attorney, Agent, or Firm* — Kirton McConkie; Evan R. Witt

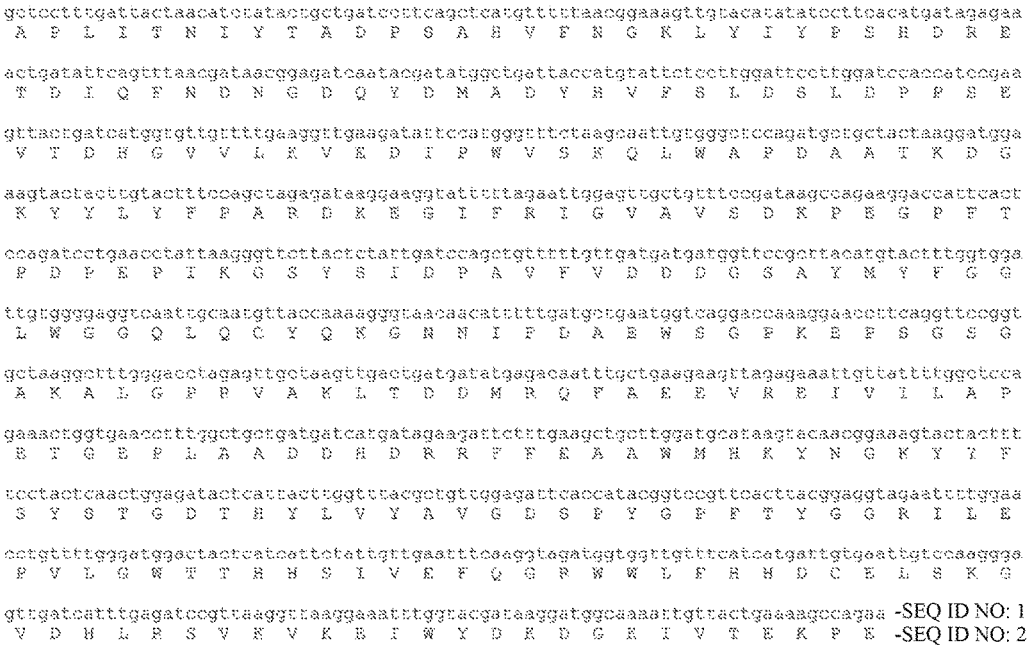
(57) **ABSTRACT**

A xylosidase having improved enzymatic activity is disclosed. The amino acid sequence of the xylosidase is a modified amino acid sequence of SEQ ID NO: 2, wherein the modification is a substitution of phenylalanine at position 35 with glutamate, and/or a substitution of glutamine at position 41 with histidine.

3 Claims, 6 Drawing Sheets

Specification includes a Sequence Listing.

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V D H L R S V K V K E I W Y D K D G K I V T E K P E -SEQ ID NO: 2

FIG. 1

Mutant	Mutagenic Primer Sequence
F35E	5'-GAGAAACTGATATTCAGGAAAACGATAACGGAGATC-3' (SEQ ID NO: 3)
Q41H	5'-GTTTAACGATAACGGAGATCATTACGATATGGCTGATTACC-3' (SEQ ID NO: 4)

FIG. 2

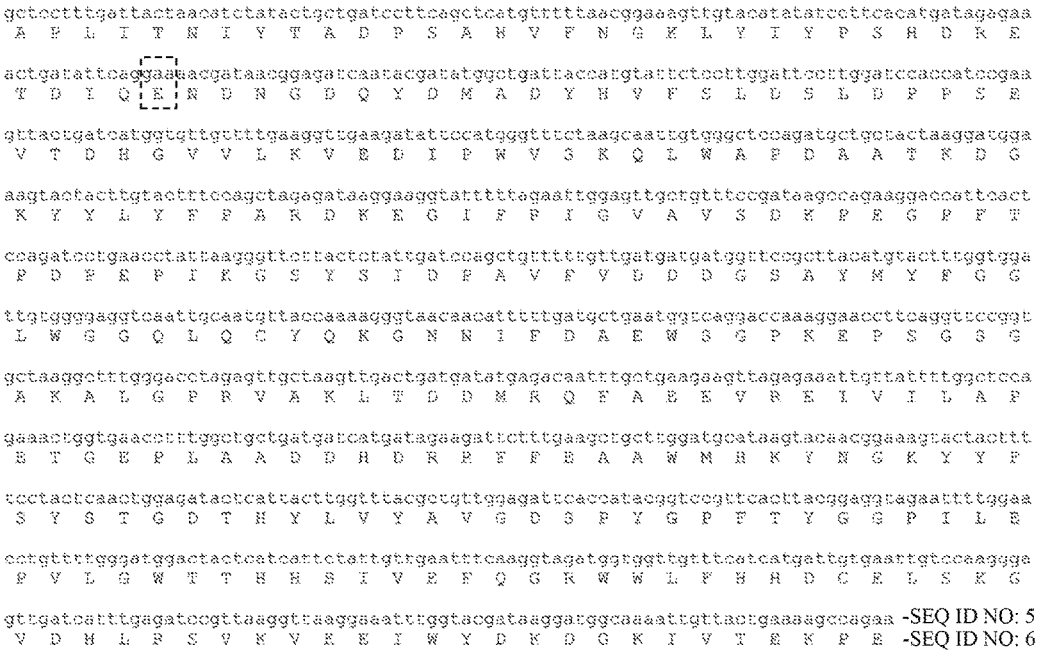
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FIG. 3

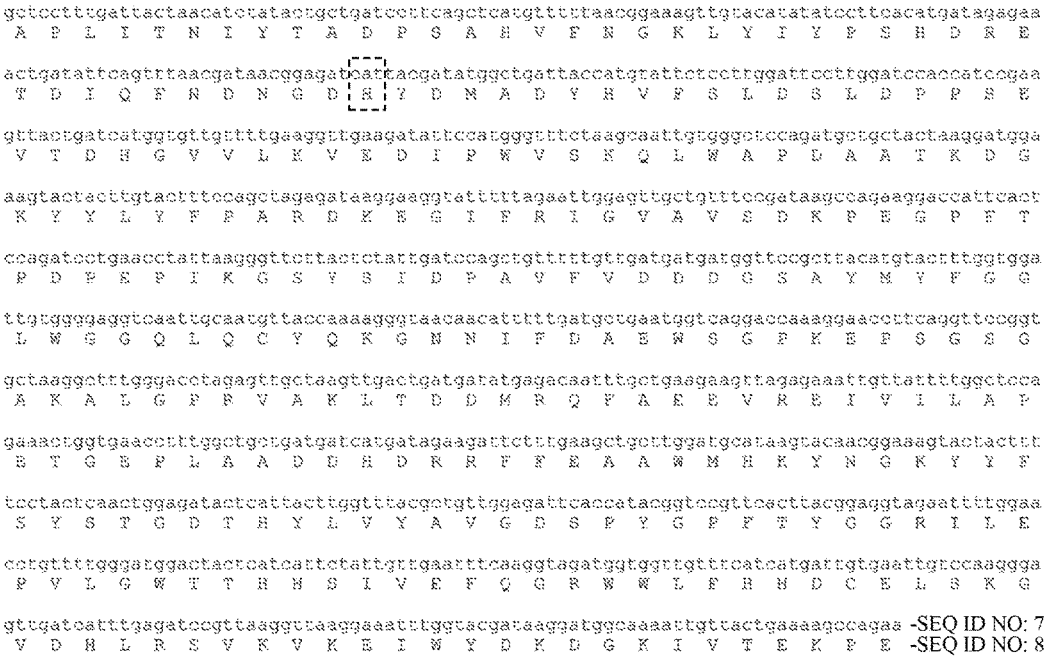
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FIG. 4

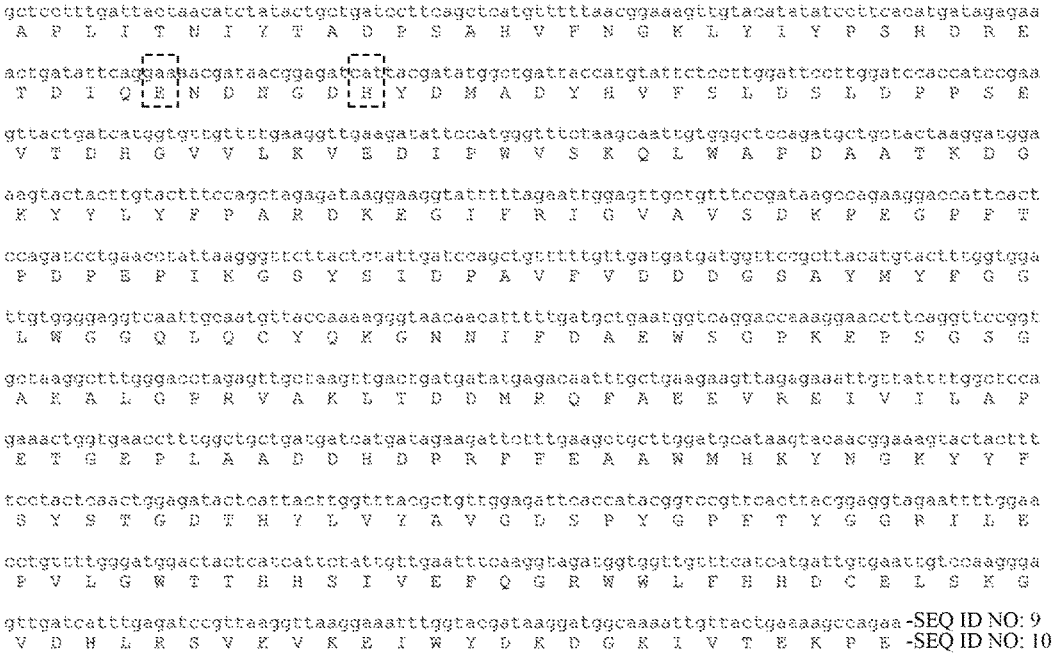

FIG. 5

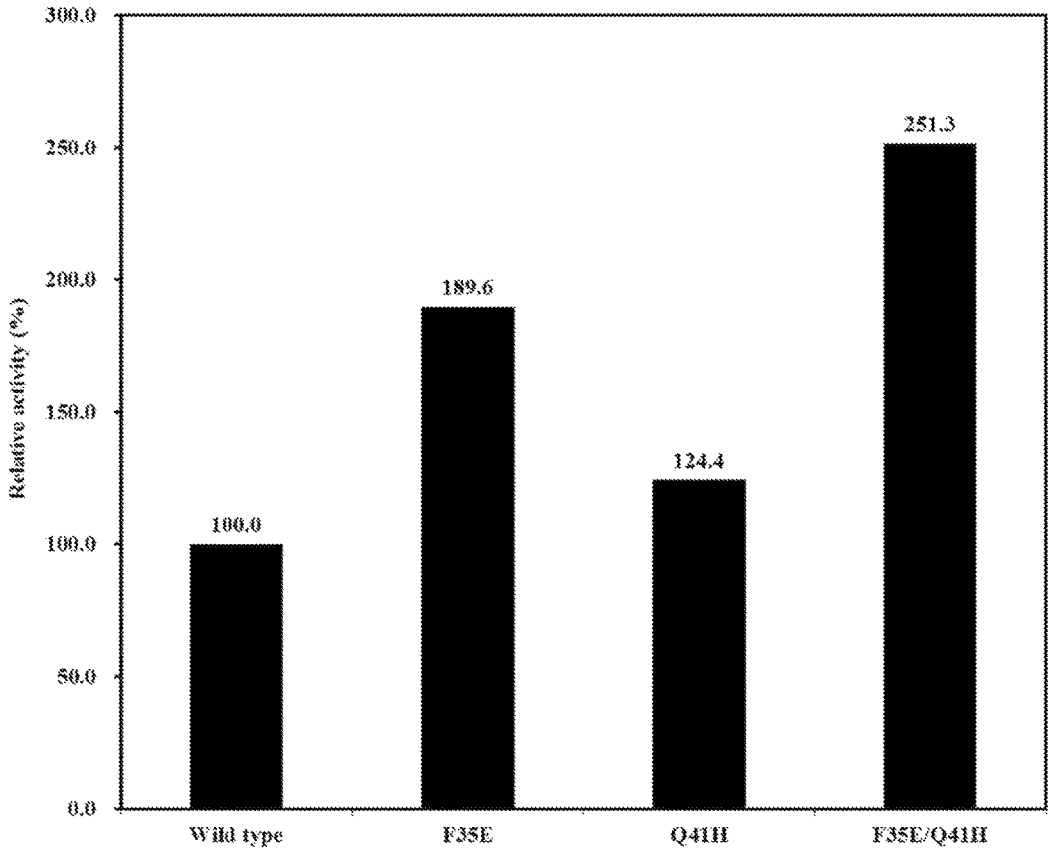
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Relative activity (%)
100.0
189.6
124.4
251.3
Wild type
F35E
Q41H
F35E/Q41H

FIG. 6

XYLOSIDASE HAVING IMPROVED ENZYMATIC ACTIVITY

FIELD OF THE INVENTION

The present invention relates to a xylosidase, and more particularly to a xylosidase having improved enzymatic activity.

BACKGROUND OF THE INVENTION

Xylan is hemicellulose, which is the major component in plant cell wall, and also the second most abundant polysaccharides on earth. Therefore, the hydrolytic enzymes that degrade xylan are highly attractive and widely applied in many industries for a long time. Xylan is a long chain polysaccharide, which is composed of many pentose xylose units linked by β-1,4-glycosidic bond as a backbone of xylan. In nature, xylan is complex and highly branched heteropolysaccharide which can be decorated by methyl group or acetyl group, even branched by other sugar molecules to form various structures of xylan. Because of this complicated architecture of xylan, the complete degradation of xylan requires different xylanolytic enzymes to work together for decomposing xylan into simple sugars that can be used by organisms.

In general, xylanolytic enzymes can be divided into several groups including endo-β-D-xylanase, β-1,4-xylosidase, arabinase, acetylxylan esterase and α-glucuronidase. Among these enzymes, β-1,4-xylosidase (EC 3.2.1.37) is a crucial enzyme for complete degradation of xylan. It is an exoglucosidase that can hydrolyze the non-reducing ends of xylooligosaccharides into simple sugar xylose.

Since the xylosidase works together with the endo-xylanase and other xylanolytic enzymes, these enzymes can be cooperatively used in many different industries, such as bleaching process in paper industry, dough quality and juice clearance in food industry, animal nutrition in feed industry, even in biofuel production. According to different industrial needs, xylosidase is required to be suitable for different appropriate working conditions. In addition to the protein properties of enzyme, its catalytic efficiency is also the key point for improving industrial enzyme. Higher enzymatic activity represents the cost reduction in the industrial process and further enhances the commercial profit.

Currently, many researches try to obtain better enzymes by either screening in nature or modifying present enzymes. In the present invention, xylosidase is modified by rational design to increase its enzymatic activity, so as to further increase its application potential and economic value in industry.

SUMMARY OF THE INVENTION

An object of the present invention is to modify a xylosidase by means of structural analysis and site-directed mutagenesis for improving the enzymatic activity of the xylosidase and further increasing its application potential and economic value in industry.

According to an aspect of the present invention, there is provided a xylosidase comprising a modified amino acid sequence of SEQ ID NO: 2, wherein the modification is a substitution of phenylalanine at position 35 with glutamate, and a substitution of glutamine at position 41 with histidine. The gene encoding the amino acid sequence of SEQ ID NO: 2 is Hixy143A gene isolated from *Humicola insolens*. The xylosidase has a full length amino acid sequence of SEQ ID NO: 10.

According to another aspect of the present invention, there is provided a xylosidase comprising a modified amino acid sequence of SEQ ID NO: 2, wherein the modification is a substitution of phenylalanine at position 35 with glutamate. The gene encoding the amino acid sequence of SEQ ID NO: 2 is Hixy143A gene isolated from *Humicola insolens*. The xylosidase has a full length amino acid sequence of SEQ ID NO: 6.

According to an additional aspect of the present invention, there is provided a xylosidase comprising a modified amino acid sequence of SEQ ID NO: 2, wherein the modification is a substitution of glutamine at position 41 with histidine. The gene encoding the amino acid sequence of SEQ ID NO: 2 is Hixy143A gene isolated from *Humicola insolens*. The xylosidase has a full length amino acid sequence of SEQ ID NO: 8.

The above objects and advantages of the present invention will become more readily apparent to those ordinarily skilled in the art after reviewing the following detailed description and accompanying drawings, in which:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the nucleotide sequence and the amino acid sequence of the wild type xylosidase Hixy143A;

FIG. **2** shows the mutagenic primer sequences for site-directed mutagenesis;

FIG. **3** shows the nucleotide sequence and the amino acid sequence of the F35E mutant;

FIG. **4** shows the nucleotide sequence and the amino acid sequence of the Q41H mutant;

FIG. **5** shows the nucleotide sequence and the amino acid sequence of the F35E/Q41H mutant; and

FIG. **6** shows the enzymatic activity analysis of the wild type Hixy143A and the three mutants.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention will now be described more specifically with reference to the following embodiments. It is to be noted that the following descriptions of preferred embodiments of this invention are presented herein for purpose of illustration and description only; it is not intended to be exhaustive or to be limited to the precise form disclosed.

The xylosidase employed in the present invention is encoded by Hixy143A gene isolated from the themophilic fungus *Humicola insolens* Y1. According to previous studies, the optimal working condition of this xylosidase is at 50° C., pH 6.8. In the present invention, the Hixy143A gene was cloned into a vector and transformed into *Pichia pastoris* for protein expression. For improving the industrial application value of this xylosidase, the present invention analyzed its protein structure and chose some potential amino acids for modifications by site-directed mutagenesis so as to improve the enzymatic activity of the xylosidase. Based on the structural analysis, Phe35 and Gln41, which are both located in the active site of the xylosidase, were chosen for further modifications. By site-directed mutagenesis, Phe35 was singly mutated to glutamate as F35E mutant, while Gln41 was singly mutated to histidine as Q41H mutant. These two mutation sites were even com-

bined into F35E/Q41H double mutant. The above mutations all successfully improved the enzymatic activity of the xylosidase.

The enzyme modification processes and the resulted xylosidase are described in detail as follows.

FIG. **1** shows the nucleotide sequence and the amino acid sequence of the wild type xylosidase Hixy143A, wherein the Hixy143A gene includes 978 base pairs (the nucleotide sequence was numbered as SEQ ID NO: 1) and encodes 326 amino acids (the amino acid sequence was numbered as SEQ ID NO: 2). First, the Hixy143A gene was cloned into pPICZαA vector by EcoRI and NotI. The plasmid DNA was linearized by PmeI and then transformed into *Pichia pastoris*. The transformants were selected by YPD plate with 0.1 mg/ml zeocin at 30° C. for 2 days. The selected clones were individually inoculated in YPD medium at 30° C. overnight and then amplified in BMGY medium at 30° C. overnight. Finally, the amplified cells were transferred to BMMY medium containing 0.5% methanol to induce the protein expression. The supernatants with induced proteins were collected by centrifugation for following analysis.

The three mutated genes of Hixy143A were obtained by site-directed mutagenesis. Particularly, these mutated sequences were obtained by polymerase chain reaction method using the wild type Hixy143A gene as the template and using the mutagenic primers shown in FIG. **2**. F35E means the phenylalanine at position 35 was substituted with glutamate, and the mutagenic primer F35E was numbered as SEQ ID NO: 3. Q41H means the glutamine at position 41 was substituted with histidine, and the mutagenic primer Q41H was numbered as SEQ ID NO: 4. Therefore, the three mutated genes of Hixy143A obtained by site-directed mutagenesis in the present invention were F35E, Q41H and F35E/Q41H.

FIGS. **3** to **5** show the nucleotide sequences and the amino acid sequences of the three mutants. FIG. **3** shows the nucleotide sequence and the amino acid sequence of the F35E mutant, wherein the nucleotide sequence was numbered as SEQ ID NO: 5, the amino acid sequence was numbered as SEQ ID NO: 6, and the phenylalanine at position 35 was substituted with glutamate. FIG. **4** shows the nucleotide sequence and the amino acid sequence of the Q41H mutant, wherein the nucleotide sequence was numbered as SEQ ID NO: 7, the amino acid sequence was numbered as SEQ ID NO: 8, and the glutamine at position 41 was substituted with histidine. FIG. **5** shows the nucleotide sequence and the amino acid sequence of the F35E/Q41H mutant, wherein the nucleotide sequence was numbered as SEQ ID NO: 9, the amino acid sequence was numbered as SEQ ID NO: 10, and the phenylalanine at position 35 was substituted with glutamate and the glutamine at position 41 was substituted with histidine.

The original DNA template was removed by DpnI at 37° C. The three mutated genes were individually transformed into *E. coli*. The success of gene mutation was confirmed by DNA sequencing. Finally, the three successful mutated genes were separately transformed into *P. pastoris* and then induced for expressing the mutated proteins by the same method mentioned above. Afterwards, the wild type protein and the mutated proteins were further analyzed for their enzymatic activity.

The xylosidase activity analysis was determined by the measurement of released nitrophenol that is a chromogenic product from the hydrolysis of the substrate p-nitrophenyl-β-D-xylopyranoside by xylosidase and further calculated to determine the enzymatic activity of xylosidase. Basically, the reaction mixture composed of diluted protein sample and 5 mM p-nitrophenyl-β-D-xylopyranoside was incubated at 50° C. for 10 min. The reaction was then stopped by using 2 M Na_2CO_3. Finally, the absorption of OD410 nm was detected to determine the activity of xylosidase.

FIG. **6** shows the enzymatic activity analysis of the wild type Hixy143A and the three mutants. As shown in FIG. **6**, under the same protein concentrations of these proteins, the single mutants F35E and Q41H both showed higher activities than did the wild type protein. When compared to the wild type protein, F35E mutant significantly increased the activity of nearly 90% while Q41H mutant increased the activity of about 20%. The double mutant F35E/Q41H showed notably increased activity to about 250%, which is much higher than the wild type protein and the single mutants. Besides, the protein expression levels of the mutants were similar to that of the wild type protein. Therefore, the total activities of these mutants F35E, Q41H and F35E/Q41H were also higher than that of the wild type protein, and the double mutant F35E/Q41H had the highest activity. It is clear that the three mutants provided in the present invention all have higher activities when compared to the wild type protein Hixy143A, that means the production cost of the xylosidase can be reduced, and thus the mutants have higher economic value of industrial application.

In conclusion, to improve the enzymatic activity of the xylosidase Hixy143A, the present invention chose some potential amino acids according to its structural analysis and further modified this enzyme by rational design. As a result, the three mutants including F35E, Q41H and F35E/Q41H all showed higher enzymatic activities compared to the wild type protein, and even had 2.5-fold increase. Therefore, the present invention successfully improves the enzymatic activity of the xylosidase and further increases its economic value of industrial application.

Nowadays, the application fields of the xylosidase and its related xylanase in industry are widespread. For feed industry, xylanolytic enzymes can be mixed with feed to help the digestion and absorption of the monogastric animals like pig and chicken by degrading feed materials with hemicellulose. As for paper and pulp industry, the enzymes also can reduce or replace the traditional toxic chemical method to reach the same result of bleaching. Besides, the enzymes provide assistances in the juice clearance and the saccharification step of brewing industry. As for biofuel production, xylosidase can degrade substrates to produce single sugars that can be utilized in fermentation by microorganisms. Thus, xylosidase can be widely used in various industries and has high economic value. The present invention modifies the xylosidase by genetic engineering, and the modified enzymes have significantly improved enzymatic activity, so the production cost of the xylosidase can be reduced to further improve the economic value of industrial application.

While the invention has been described in terms of what is presently considered to be the most practical and preferred embodiments, it is to be understood that the invention needs not be limited to the disclosed embodiment. On the contrary, it is intended to cover various modifications and similar arrangements included within the spirit and scope of the appended claims which are to be accorded with the broadest interpretation so as to encompass all such modifications and similar structures.

SEQUENCE LISTING

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<212> TYPE: DNA
<213> ORGANISM: Humicola insolens

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Ile Gln Phe Asn Asp Asn Gly Asp Gln Tyr Asp Met Ala Asp Tyr His
35 40 45

Val Phe Ser Leu Asp Ser Leu Asp Pro Pro Ser Glu Val Thr Asp His
50 55 60

Gly Val Val Leu Lys Val Glu Asp Ile Pro Trp Val Ser Lys Gln Leu
65 70 75 80

Trp Ala Pro Asp Ala Ala Thr Lys Asp Gly Lys Tyr Tyr Leu Tyr Phe
85 90 95

Pro Ala Arg Asp Lys Glu Gly Ile Phe Arg Ile Gly Val Ala Val Ser
100 105 110

Asp Lys Pro Glu Gly Pro Phe Thr Pro Asp Pro Glu Pro Ile Lys Gly
115 120 125

Ser Tyr Ser Ile Asp Pro Ala Val Phe Val Asp Asp Asp Gly Ser Ala
130 135 140

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Tyr Met Tyr Phe Gly Gly Leu Trp Gly Gly Gln Leu Gln Cys Tyr Gln
145                 150                 155                 160

Lys Gly Asn Asn Ile Phe Asp Ala Glu Trp Ser Gly Pro Lys Glu Pro
                165                 170                 175

Ser Gly Ser Gly Ala Lys Ala Leu Gly Pro Arg Val Ala Lys Leu Thr
            180                 185                 190

Asp Asp Met Arg Gln Phe Ala Glu Glu Val Arg Glu Ile Val Ile Leu
        195                 200                 205

Ala Pro Glu Thr Gly Glu Pro Leu Ala Ala Asp Asp His Asp Arg Arg
    210                 215                 220

Phe Phe Glu Ala Ala Trp Met His Lys Tyr Asn Gly Lys Tyr Tyr Phe
225                 230                 235                 240

Ser Tyr Ser Thr Gly Asp Thr His Tyr Leu Val Tyr Ala Val Gly Asp
                245                 250                 255

Ser Pro Tyr Gly Pro Phe Thr Tyr Gly Gly Arg Ile Leu Glu Pro Val
            260                 265                 270

Leu Gly Trp Thr Thr His His Ser Ile Val Glu Phe Gln Gly Arg Trp
        275                 280                 285

Trp Leu Phe His His Asp Cys Glu Leu Ser Lys Gly Val Asp His Leu
    290                 295                 300

Arg Ser Val Lys Val Lys Glu Ile Trp Tyr Asp Lys Asp Gly Lys Ile
305                 310                 315                 320

Val Thr Glu Lys Pro Glu
                325
```

<210> SEQ ID NO 3
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetically generated primer

<400> SEQUENCE: 3

gagaaactga tattcaggaa aacgataacg gagatc 36

<210> SEQ ID NO 4
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetically generated primer

<400> SEQUENCE: 4

gtttaacgat aacggagatc attacgatat ggctgattac c 41

<210> SEQ ID NO 5
<211> LENGTH: 978
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetically generated DNA encoding a modified enzyme

<400> SEQUENCE: 5

gctcctttga ttactaacat ctatactgct gatccttcag ctcatgtttt taacggaaag 60

ttgtacatat atccttcaca tgatagagaa actgatattc aggaaaacga taacggagat 120

caatacgata tggctgatta ccatgtattc tccttggatt ccttggatcc accatccgaa 180

gttactgatc atggtgttgt tttgaaggtt gaagatattc catgggtttc taagcaattg 240

-continued

tgggctccag atgctgctac taaggatgga aagtactact tgtactttcc agctagagat 300

aaggaaggta tttttagaat tggagttgct gtttccgata agccagaagg accattcact 360

ccagatcctg aacctattaa gggttcttac tctattgatc cagctgtttt tgttgatgat 420

gatggttccg cttacatgta ctttggtgga ttgtggggag gtcaattgca atgttaccaa 480

aagggtaaca acatttttga tgctgaatgg tcaggaccaa aggaaccttc aggttccggt 540

gctaaggctt tgggacctag agttgctaag ttgactgatg atatgagaca atttgctgaa 600

gaagttagag aaattgttat tttggctcca gaaactggtg aacctttggc tgctgatgat 660

catgatagaa gattctttga agctgcttgg atgcataagt acaacggaaa gtactacttt 720

tcctactcaa ctggagatac tcattacttg gtttacgctg ttggagattc accatacggt 780

ccgttcactt acggaggtag aattttggaa cctgttttgg gatggactac tcatcattct 840

attgttgaat ttcaaggtag atggtggttg tttcatcatg attgtgaatt gtccaaggga 900

gttgatcatt tgagatccgt taaggttaag gaaatttggt acgataagga tggcaaaatt 960

gttactgaaa agccagaa 978

<210> SEQ ID NO 6
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sequence synthetically translated from SEQ ID NO: 5

<400> SEQUENCE: 6

Ala Pro Leu Ile Thr Asn Ile Tyr Thr Ala Asp Pro Ser Ala His Val
1 5 10 15

Phe Asn Gly Lys Leu Tyr Ile Tyr Pro Ser His Asp Arg Glu Thr Asp
20 25 30

Ile Gln Glu Asn Asp Asn Gly Asp Gln Tyr Asp Met Ala Asp Tyr His
35 40 45

Val Phe Ser Leu Asp Ser Leu Asp Pro Pro Ser Glu Val Thr Asp His
50 55 60

Gly Val Val Leu Lys Val Glu Asp Ile Pro Trp Val Ser Lys Gln Leu
65 70 75 80

Trp Ala Pro Asp Ala Ala Thr Lys Asp Gly Lys Tyr Tyr Leu Tyr Phe
85 90 95

Pro Ala Arg Asp Lys Glu Gly Ile Phe Arg Ile Gly Val Ala Val Ser
100 105 110

Asp Lys Pro Glu Gly Pro Phe Thr Pro Asp Pro Glu Pro Ile Lys Gly
115 120 125

Ser Tyr Ser Ile Asp Pro Ala Val Phe Val Asp Asp Asp Gly Ser Ala
130 135 140

Tyr Met Tyr Phe Gly Gly Leu Trp Gly Gly Gln Leu Gln Cys Tyr Gln
145 150 155 160

Lys Gly Asn Asn Ile Phe Asp Ala Glu Trp Ser Gly Pro Lys Glu Pro
165 170 175

Ser Gly Ser Gly Ala Lys Ala Leu Gly Pro Arg Val Ala Lys Leu Thr
180 185 190

Asp Asp Met Arg Gln Phe Ala Glu Glu Val Arg Glu Ile Val Ile Leu
195 200 205

Ala Pro Glu Thr Gly Glu Pro Leu Ala Ala Asp Asp His Asp Arg Arg
210 215 220

-continued

```
Phe Phe Glu Ala Ala Trp Met His Lys Tyr Asn Gly Lys Tyr Tyr Phe
225                 230                 235                 240

Ser Tyr Ser Thr Gly Asp Thr His Tyr Leu Val Tyr Ala Val Gly Asp
                245                 250                 255

Ser Pro Tyr Gly Pro Phe Thr Tyr Gly Gly Arg Ile Leu Glu Pro Val
            260                 265                 270

Leu Gly Trp Thr Thr His His Ser Ile Val Glu Phe Gln Gly Arg Trp
        275                 280                 285

Trp Leu Phe His His Asp Cys Glu Leu Ser Lys Gly Val Asp His Leu
    290                 295                 300

Arg Ser Val Lys Val Lys Glu Ile Trp Tyr Asp Lys Asp Gly Lys Ile
305                 310                 315                 320

Val Thr Glu Lys Pro Glu
                325
```

<210> SEQ ID NO 7
<211> LENGTH: 978
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetically generated DNA encoding a modified enzyme

<400> SEQUENCE: 7

```
gctcctttga ttactaacat ctatactgct gatccttcag ctcatgtttt taacggaaag     60

ttgtacatat atccttcaca tgatagagaa actgatattc agtttaacga taacggagat    120

cattacgata tggctgatta ccatgtattc tccttggatt ccttggatcc accatccgaa    180

gttactgatc atggtgttgt tttgaaggtt gaagatattc catgggtttc taagcaattg    240

tgggctccag atgctgctac taaggatgga aagtactact tgtactttcc agctagagat    300

aaggaaggta tttttagaat tggagttgct gtttccgata agccagaagg accattcact    360

ccagatcctg aacctattaa gggttcttac tctattgatc cagctgtttt tgttgatgat    420

gatggttccg cttacatgta ctttggtgga ttgtggggag gtcaattgca atgttaccaa    480

aagggtaaca acatttttga tgctgaatgg tcaggaccaa aggaaccttc aggttccggt    540

gctaaggctt tgggacctag agttgctaag ttgactgatg atatgagaca atttgctgaa    600

gaagttagag aaattgttat tttggctcca gaaactggtg aacctttggc tgctgatgat    660

catgatagaa gattctttga agctgcttgg atgcataagt acaacggaaa gtactacttt    720

tcctactcaa ctggagatac tcattacttg gtttacgctg ttggagattc accatacggt    780

ccgttcactt acggaggtag aattttggaa cctgttttgg gatggactac tcatcattct    840

attgttgaat ttcaaggtag atggtggttg tttcatcatg attgtgaatt gtccaaggga    900

gttgatcatt tgagatccgt taaggttaag gaaatttggt acgataagga tggcaaaatt    960

gttactgaaa agccagaa                                                  978
```

<210> SEQ ID NO 8
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sequence synthetically translated from SEQ ID NO: 7

<400> SEQUENCE: 8

```
Ala Pro Leu Ile Thr Asn Ile Tyr Thr Ala Asp Pro Ser Ala His Val
1               5                   10                  15
```

-continued

```
Phe Asn Gly Lys Leu Tyr Ile Tyr Pro Ser His Asp Arg Glu Thr Asp
            20                  25                  30

Ile Gln Phe Asn Asp Asn Gly Asp His Tyr Asp Met Ala Asp Tyr His
        35                  40                  45

Val Phe Ser Leu Asp Ser Leu Asp Pro Pro Ser Glu Val Thr Asp His
    50                  55                  60

Gly Val Val Leu Lys Val Glu Asp Ile Pro Trp Val Ser Lys Gln Leu
65                  70                  75                  80

Trp Ala Pro Asp Ala Ala Thr Lys Asp Gly Lys Tyr Tyr Leu Tyr Phe
                85                  90                  95

Pro Ala Arg Asp Lys Glu Gly Ile Phe Arg Ile Gly Val Ala Val Ser
            100                 105                 110

Asp Lys Pro Glu Gly Pro Phe Thr Pro Asp Pro Glu Pro Ile Lys Gly
        115                 120                 125

Ser Tyr Ser Ile Asp Pro Ala Val Phe Val Asp Asp Asp Gly Ser Ala
    130                 135                 140

Tyr Met Tyr Phe Gly Gly Leu Trp Gly Gly Gln Leu Gln Cys Tyr Gln
145                 150                 155                 160

Lys Gly Asn Asn Ile Phe Asp Ala Glu Trp Ser Gly Pro Lys Glu Pro
                165                 170                 175

Ser Gly Ser Gly Ala Lys Ala Leu Gly Pro Arg Val Ala Lys Leu Thr
            180                 185                 190

Asp Asp Met Arg Gln Phe Ala Glu Glu Val Arg Glu Ile Val Ile Leu
        195                 200                 205

Ala Pro Glu Thr Gly Glu Pro Leu Ala Ala Asp Asp His Asp Arg Arg
    210                 215                 220

Phe Phe Glu Ala Ala Trp Met His Lys Tyr Asn Gly Lys Tyr Tyr Phe
225                 230                 235                 240

Ser Tyr Ser Thr Gly Asp Thr His Tyr Leu Val Tyr Ala Val Gly Asp
                245                 250                 255

Ser Pro Tyr Gly Pro Phe Thr Tyr Gly Gly Arg Ile Leu Glu Pro Val
            260                 265                 270

Leu Gly Trp Thr Thr His His Ser Ile Val Glu Phe Gln Gly Arg Trp
        275                 280                 285

Trp Leu Phe His His Asp Cys Glu Leu Ser Lys Gly Val Asp His Leu
    290                 295                 300

Arg Ser Val Lys Val Lys Glu Ile Trp Tyr Asp Lys Asp Gly Lys Ile
305                 310                 315                 320

Val Thr Glu Lys Pro Glu
                325
```

<210> SEQ ID NO 9
<211> LENGTH: 978
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetically generated DNA encoding a modified enzyme

<400> SEQUENCE: 9

```
gctcctttga ttactaacat ctatactgct gatccttcag ctcatgtttt taacggaaag     60

ttgtacatat atccttcaca tgatagagaa actgatattc aggaaaacga taacggagat    120

cattacgata tggctgatta ccatgtattc tccttggatt ccttggatcc accatccgaa    180

gttactgatc atggtgttgt tttgaaggtt gaagatattc catgggtttc taagcaattg    240
```

-continued

tgggctccag atgctgctac taaggatgga aagtactact tgtactttcc agctagagat 300

aaggaaggta tttttagaat tggagttgct gtttccgata agccagaagg accattcact 360

ccagatcctg aacctattaa gggttcttac tctattgatc cagctgtttt tgttgatgat 420

gatggttccg cttacatgta ctttggtgga ttgtggggag gtcaattgca atgttaccaa 480

aagggtaaca acatttttga tgctgaatgg tcaggaccaa aggaaccttc aggttccggt 540

gctaaggctt tgggacctag agttgctaag ttgactgatg atatgagaca atttgctgaa 600

gaagttagag aaattgttat tttggctcca gaaactggtg aacctttggc tgctgatgat 660

catgatagaa gattctttga agctgcttgg atgcataagt acaacggaaa gtactacttt 720

tcctactcaa ctggagatac tcattacttg gtttacgctg ttggagattc accatacggt 780

ccgttcactt acggaggtag aattttggaa cctgttttgg gatggactac tcatcattct 840

attgttgaat ttcaaggtag atggtggttg tttcatcatg attgtgaatt gtccaaggga 900

gttgatcatt tgagatccgt taaggttaag gaaatttggt acgataagga tggcaaaatt 960

gttactgaaa agccagaa 978

<210> SEQ ID NO 10
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Sequence synthetically translated from SEQ ID NO: 9

<400> SEQUENCE: 10

Ala Pro Leu Ile Thr Asn Ile Tyr Thr Ala Asp Pro Ser Ala His Val
1 5 10 15

Phe Asn Gly Lys Leu Tyr Ile Tyr Pro Ser His Asp Arg Glu Thr Asp
20 25 30

Ile Gln Glu Asn Asp Asn Gly Asp His Tyr Asp Met Ala Asp Tyr His
35 40 45

Val Phe Ser Leu Asp Ser Leu Asp Pro Pro Ser Glu Val Thr Asp His
50 55 60

Gly Val Val Leu Lys Val Glu Asp Ile Pro Trp Val Ser Lys Gln Leu
65 70 75 80

Trp Ala Pro Asp Ala Ala Thr Lys Asp Gly Lys Tyr Tyr Leu Tyr Phe
85 90 95

Pro Ala Arg Asp Lys Glu Gly Ile Phe Arg Ile Gly Val Ala Val Ser
100 105 110

Asp Lys Pro Glu Gly Pro Phe Thr Pro Asp Pro Glu Pro Ile Lys Gly
115 120 125

Ser Tyr Ser Ile Asp Pro Ala Val Phe Val Asp Asp Asp Gly Ser Ala
130 135 140

Tyr Met Tyr Phe Gly Gly Leu Trp Gly Gly Gln Leu Gln Cys Tyr Gln
145 150 155 160

Lys Gly Asn Asn Ile Phe Asp Ala Glu Trp Ser Gly Pro Lys Glu Pro
165 170 175

Ser Gly Ser Gly Ala Lys Ala Leu Gly Pro Arg Val Ala Lys Leu Thr
180 185 190

Asp Asp Met Arg Gln Phe Ala Glu Glu Val Arg Glu Ile Val Ile Leu
195 200 205

Ala Pro Glu Thr Gly Glu Pro Leu Ala Ala Asp Asp His Asp Arg Arg
210 215 220

-continued

```
Phe Phe Glu Ala Ala Trp Met His Lys Tyr Asn Gly Lys Tyr Tyr Phe
225                 230                 235                 240

Ser Tyr Ser Thr Gly Asp Thr His Tyr Leu Val Tyr Ala Val Gly Asp
                245                 250                 255

Ser Pro Tyr Gly Pro Phe Thr Tyr Gly Gly Arg Ile Leu Glu Pro Val
            260                 265                 270

Leu Gly Trp Thr Thr His His Ser Ile Val Glu Phe Gln Gly Arg Trp
        275                 280                 285

Trp Leu Phe His His Asp Cys Glu Leu Ser Lys Gly Val Asp His Leu
    290                 295                 300

Arg Ser Val Lys Val Lys Glu Ile Trp Tyr Asp Lys Asp Gly Lys Ile
305                 310                 315                 320

Val Thr Glu Lys Pro Glu
                325
```

What is claimed is:

1. A xylosidase consisting of a modified amino acid sequence of SEQ ID NO: 2, wherein the modification is a substitution of phenylalanine at position 35 with glutamate, and a substitution of glutamine at position 41 with histidine.

2. A xylosidase consisting of a modified amino acid sequence of SEQ ID NO: 2, wherein the modification is a substitution of phenylalanine at position 35 with glutamate.

3. A xylosidase consisting of a modified amino acid sequence of SEQ ID NO: 2, wherein the modification is a substitution of glutamine at position 41 with histidine.

* * * * *