Item	Value
Enzyme	Trypsin, Chymotrypsin, Glu-C
modifications	Deamidated(NQ), Oxidation(M), Carbamidomethyl(C)
M/Z tolerance	±15ppm
Fragment tolerance	Fragment tolerance
Database	20200604366.fasta
Filter	Minimum fragment ions:3

[0098] 4 Experimental results and analysis

[0099] After the test samples were enzymatically digested with Trypsin, Chymotrypsin, and Glu-C respectively in solution, the peptide samples were analyzed by LC-MS/MS equipment, and the raw data obtained was queried by UNIFI software.

[0100] 1) The molecular weight of P-012 and the corresponding polypeptide detected by mass spectrometer.

Observed value	Mr (Expected value)	Peptide
1171.2805	1170.1811	GERGAPGFRGPA (SEQ ID NO: 6)
1660.8642	1659.8569	GPAGPNGIPGEKGPAGER (SEQ ID NO: 7)
1678.8994	1677.8921	GAPGFRGPAGPNGIPGEK (SEQ ID NO: 8)
2244.0007	2242.9934	GPAGPNGIPGEKGPAGERGAPGER (SEQ ID NO: 9)
2244.3176	2243.3104	GPAGPNGIPGEKGPAGERGAPGER (SEQ ID NO: 9)
2246.2154	2245.2081	GAPGFRGPAGPNGIPGEKGPAGER (SEQ ID NO: 10)

[0101] The coverage of the detected polypeptide fragment was 100%.

[0102] 2) The molecular weight of P-C3T8 and the corresponding polypeptide detected by mass spectrometer.

Observed value	Mr (Expected value)	Peptide
1706.8815	1705.5321	GERGAPGFRGPAGPNGIP (SEQ ID NO: 11)
1093.5637	1092.5564	GPAGPNGIPGEK (SEQ ID NO: 12)
1660.8636	1659.8563	GPAGPNGIPGEKGPAGER (SEQ ID NO: 7)

1678.8907	1677.8834	GAPGFRGPAGPNGIPGEK (SEQ ID NO: 8)
2244.3176	2243.3104	GPAGPNGIPGEKGPAGERGAPGER (SEQ ID NO: 9)
2246.1276	2245.1203	GAPGFRGPAGPNGIPGEKGPAGER (SEQ ID NO: 10)

[0103] The coverage of the detected polypeptide fragment was 100%.

[0104] After the enzymatically digested samples were analyzed by LC-MS/MS, the query results were integrated. The peptide coverage of the final samples obtained was 100%, and the detection results were very reliable.

Example 4: Detection of cell adhesion activity of recombinant humanized type III collagens

[0105] The method for detecting the activity of collagen can refer to the literature Juming Yao, Satoshi Yanagisawa, Tetsuo Asakura, Design, Expression and Characterization of Collagen-Like Proteins Based on the Cell Adhesive and Crosslinking Sequences Derived from Native Collagens, J Biochem. 136, 643-649 (2004). The specific implementation method was as follows:

[0106] (1) The concentration of the protein sample to be tested, including bovine type I collagen (China Food and Drug Inspection Institute, No.: 380002) and the recombinant humanized protein provided by the present disclosure was detected by ultraviolet absorption method. Specifically, the ultraviolet absorption of the sample at 215 nm and 225 nm was measured respectively, and the protein concentration was calculated using the empirical formula C (μg/mL) = 144 x (A215-A225). Note that the detection needs to be performed when A215 < 1.5. The principle of this method is to measure the characteristic absorption of peptide bonds under far-ultraviolet light, which is not affected by the chromophore content. The interfering substances are less in the method. The method is easy to operate, and is suitable for detecting human collagen and its analogs that are not stained with Coomassie Brilliant Blue. (Reference: Walker JM. The Protein Protocols Handbook, second edition. Humana Press. 43-45.). After the protein concentration was detected, the concentration of all proteins to be tested was adjusted to 0.5 mg/mL with PBS.

[0107] (2) The 96-well plate was added with 100 μL of various protein solutions

CLAIMS

1. A method for constructing a yeast cell expressing collagen, comprising introducing a recombinant expression vector pPICZα A into the yeast cell, wherein the recombinant expression vector comprises a polynucleotide encoding the collagen, wherein the amino acid sequence of the collagen is the amino acid sequence of SEQ ID No. 2 or 3, and the yeast is *Pichia pastoris*.

2. The method according to claim 1, wherein the collagen is a recombinant type III humanized collagen.

3. The method according to claim 1, wherein the collagen is a collagen having a 164.88° triple helix structure.

4. The method according to claim 1, wherein the polynucleotide comprises a nucleotide sequence of SEQ ID No. 4.

5. The method according to claim 1, wherein the polynucleotide comprises a nucleotide sequence of SEQ ID No. 5.

6. The method according to any one of claims 1-5, wherein *Pichia pastoris* is *Pichia pastoris* X33, GS115, SMD1168, KM71 or KM71H strain.

7. The method according to any one of claims 1-5, comprising one or more of the following steps:

(1) inserting the polynucleotide into pPICZα A expression vector, between NotI and XhoI restriction sites to obtain a recombinant expression plasmid;

(2) enzymatically digesting the recombinant expression plasmid;

(3) purifying the enzymatically digested recombinant expression plasmid;

(4) introducing the enzymatically digested recombinant expression plasmid into the yeast cell by electroporation.

8. The method according to claim 7, wherein the recombinant expression plasmid is enzymatically digested with sac1.

9. A yeast cell expressing collagen, wherein the amino acid sequence of the collagen is the amino acid sequence of SEQ ID No. 2 or 3; the yeast cell is *Pichia pastoris*; the yeast cell comprises the yeast expression system vector pPICZα A and the expression vector comprises a polynucleotide comprising the nucleotide

sequence of SEQ ID NO. 4 or 5.

10. The yeast cell according to claim 9, wherein the yeast is Pichia pastoris X33, GS115, SMD1168, KM71 or KM71H strain.

11. Use of the yeast cell according to any one of claims 9-12 for preparing collagen of the amino acid sequence of SEQ ID No. 2 or 3.

12. A method for producing collagen by fermentation, comprising culturing the yeast cell according to any one of claims 9-12 under a suitable condition, adding methanol to the yeast cell to induce expression, and harvesting the culture supernatant to obtain the collagen of the amino acid sequence of SEQ ID No. 2 or 3.

13. The method according to claim 12, wherein the culture medium is a BMMY medium supplemented with biotin and a yeast nitrogen base without amino acids.

14. The method according to claim 12 or 13, comprising:

(1) culturing a seed liquid, wherein the seed liquid is the seed liquid of the yeast cell of any one of claims 9-12;

(2) fermentation culture: inoculating the seed liquid into a fermentation medium, culturing the medium in a fermenter, and starting to add methanol in a batch-fed course at a suitable growth stage to induce expression;

(3) preparation of a fermentation supernatant: culturing the medium until a suitable time and terminating the culturing, taking the fermentation liquid and removing the yeast cells, filtering the fermentation liquid, and obtaining the fermentation supernatant.

15. The method according to claim 14, wherein the collagen is a collagen having a 164.88° triple helix structure.