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Research on the Identification of Angelica Sinensis by PCR + Sequencing Based on ITS2

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Abstract

The adulteration of Chinese medicinal materials has become an important obstacle to the development of traditional Chinese medicine, and it is urgent to establish identification methods for Chinese medicinal materials. This project aims to establish a method for identifying the authenticity of Angelica sinensis based on the ITS2 gene. By extracting Angelica sinensis DNA, performing PCR amplification, adding an A-tail, nucleic acid electrophoresis, gel recovery, constructing a vector, transformation and blue white screening, plasmid extraction, PCR and enzyme digestion detection, and finally sequencing, the authenticity of Angelica sinensis samples is identified through gene library comparison. The identification results of 8 Angelica sinensis samples showed that 4 were Angelica sinensis, 3 were Angelica dahurica, and 1 was Kitagawia terebinthacea. The similarity between the ITS2 fragment and the gene library was 98.99%, 95.56%, and 98.99%, respectively. The gene identification method established in this study can accurately identify the authenticity of Angelica sinensis samples and provide a reference for Angelica sinensis identification.

Keywords

Angelica Sinensis, ITS2, PCR, Cloning, Nucleic Acid Comparison

1. Introduction

Traditional Chinese medicine is a treasure of Chinese traditional culture, and authenticity identification has been an important topic in the research of Chinese medicinal materials from ancient times to the present. The identification methods of traditional Chinese medicinal materials [1] have been continuously improved and innovated, ranging from sensory experience based identification methods to

classical identification methods using instruments and reagents [2] [3]. With the development of modern technology, the usage methods and sources of traditional Chinese medicine have become increasingly complex. There are more and more phenomena of mixing, replacing, passing off fake as genuine, and passing off inferior as good. As the saying goes, "the devil is one foot high, God is ten feet high." Therefore, the identification methods of traditional Chinese medicine should also be improved and innovated. At present, the identification methods of traditional Chinese medicinal materials mainly include morphological identification, microscopic identification, physicochemical identification, etc. [2], but these traditional methods have certain shortcomings. Recently, molecular biology and population genetics have made progress in molecular systematics, and more and more scientists are trying to apply them to the identification of modern Chinese medicinal materials. The genetic method can achieve species identification through gene fragments and has been applied in the field of traditional Chinese medicine identification. It is an effective supplement to traditional methods and avoids the limitations of morphological identification. This method can directly provide rich evidence at the genetic level. With the increasingly widespread use of traditional Chinese medicine, unscrupulous merchants, in order to seek profits, adulterate and forge Chinese medicinal materials, pass off inferior ones as good, forge their place of origin, and use low-priced Chinese medicinal materials to counterfeit high priced species or varieties. Consumers' attention to the origin and authenticity of Chinese medicinal materials is increasing. The adulteration of traditional Chinese medicinal materials has a wide range and diverse forms, and conventional methods are difficult to detect. Cutting edge technological methods such as spectral analysis technology [4], near-infrared spectroscopy analysis technology [5], traditional Chinese medicine characteristic map technology [6], high-throughput DNA sequencing technology, and nucleic acid detection [7] [8] have been used to distinguish the authenticity of traditional Chinese medicinal materials.

Angelica sinensis, a plant in the Umbelliferae family, has a sweet and pungent taste and is the dried root of Angelica sinensis (Oliv) Diels [9]. It is a traditional medicinal and edible species in China. The main production area is in the southeastern part of Gansu, with Min County having the highest yield and the best quality, followed by Yunnan, Sichuan and other provinces. It has the effects of nourishing blood and promoting blood circulation, regulating menstruation and relieving pain, moistening the intestines and promoting bowel movements [10]. Commonly used for dizziness, palpitations, menstrual disorders, etc. The identification of Angelica sinensis has also made great progress. In 2019, Fu Hong proposed using identification methods such as morphology, microscopy, and thin-layer chromatography to compare and analyze the medicinal materials and decoction pieces of Angelica sinensis, European Angelica sinensis, and Japanese Angelica sinensis [11]. In 2020, Che Surong *et al.* [12] proposed using UV spectroscopy to compare and identify the UV absorption spectra of Angelica sinensis and its mixed products Duhuo and EuAngelica sinensis in different polar solvents. In

2021, Shi Zhongfei *et al.* [13] [14] established a method based on site-specific PCR technology to quickly identify pseudo Angelica sinensis in Angelica sinensis medicinal materials and decoction pieces. Li Bo *et al.* [15] analyzed the differences in the material basis of low-temperature dried Angelica sinensis medicinal materials, decoction pieces, and standard decoctions based on HPLC fingerprint feature correlation O2PLS-DA model.

This study establishes a PCR and sequencing method for identifying Angelica sinensis based on ITS2 sequences [16]-[18]. Firstly, extract DNA from the Angelica sinensis sample, perform PCR amplification, add A tail, then perform nucleic acid electrophoresis, gel recovery, construct vector, transformation and blue white screening, plasmid extraction, PCR and enzyme digestion detection. Store and send the bacterial solution for detection sequence, compare the sequencing results with the gene library to identify the authenticity of the Angelica sinensis sample. This method will provide a new detection method for the identification of Angelica sinensis.

2. Materials and Methods

2.1. Materials and Reagents

The 7 groups of Angelica sinensis samples were purchased online (named X1 for Shangri La, L1 for Leshan City, and M1 to M5 for Min County), and the 8 groups of Angelica sinensis samples provided by the laboratory were named M6 for Min County.

Plant Genomic DNA Kit (centrifugal column type) and 10xTaq Buffer Taq DNA Polymerase, Marker III, Tiangen Biochemical Technology (Beijing) Co., Ltd; Sensory *E. coli* DH5 *α*, Biogenic Column DNA Gel Recovery Kit, Shanghai Biogenic Engineering Co., Ltd; ITS2 universal primers were synthesized by Universal Biotech (Anhui) Co., Ltd; The reagents used for PCR amplification, addition of A, and vector construction were purchased from Baoriyi Biotechnology Co., Ltd; Chloroform, anhydrous ethanol, isopropanol, etc. are analytical grade, produced by Beijing Dingguo Changsheng Biotechnology Co., Ltd.

2.2. Instruments and Equipment

DH300 thermostat, Hangzhou Ruicheng Instrument Co., Ltd; D2012 Plus Centrifuge, Dalong Xingchuang Experimental Instrument (Beijing) Co., Ltd; HS-1112B double-layer shaker, Changzhou Putian Instrument Manufacturing Co., Ltd; SW-CJ-1D ultra clean workbench, Shanghai Jianli Instrument Co., Ltd; C0 PCR amplifier, Xinyi Manufacturing Technology (Beijing) Co., Ltd; DYY-10C electrophoresis instrument, Beijing Liuyi Instrument Factory; Gel imaging system, Thermo Fisher Scientific, USA; Nano Drop One Ultra Trace Nucleic Acid Protein Analyzer, Thermo Fisher Scientific, USA.

2.3. Experimental Methods

2.3.1. Extraction of Angelica DNA

Pre treatment: Gently wipe the surface of Angelica sinensis with 75% ethanol to

remove dust, place it in a well ventilated area or blow it with a hair dryer until the anhydrous ethanol evaporates completely. Take some dried herbs and grind them into powder in a high-speed grinder.

Extraction of Angelica DNA: Weigh 30 mg of ground Angelica powder and use the plant DNA extraction kit from Beijing Tiangen Company to extract DNA (follow the instructions). Measure the concentration and purity of the extracted DNA using an ultra trace nucleic acid protein analyzer, and store the prepared sample at −20˚C for future use.

2.3.2. PCR Amplification and A-Tail Addition

PCR amplification: Angelica sinensis, as a traditional Chinese medicine, undergoes a series of processing treatments such as exposure to sunlight and drying, as well as poor storage conditions or prolonged storage, resulting in varying degrees of DNA degradation and affecting the efficiency of PCR amplification. Therefore, this study selected ITS2 fragments with high amplification efficiency and strong universality in the identification of traditional Chinese medicine. Using a 25 μL PCR reaction system: PrimeSTAR Max Premix (2X) 12.5 μL, ddH_2O 8.5 μL, Total DNA 2 μL, 1 μL each for forward and reverse primers (10 μM). Put it into the PCR amplifier and set the reaction program as follows: denaturation at 98˚C for 5 seconds, annealing at 53˚C for 10 seconds, and extension at 72˚C for 5 seconds in one cycle, with 30 cycles.

Add A suffix: For the convenience of subsequent TA cloning experiments, add A at the end of the DNA. Take out the sample from the PCR amplifier and add: dATP 0.5 μL, Taq enzyme 0.5 μL, 10 × Buffer 2.5 μL. Put the new system with a total volume of 28.5 μL back into the PCR amplifier and react at 72˚C for 30 min.

2.3.3. Nucleic Acid Electrophoresis and Gel Cutting

To prepare agarose gel: weigh 0.3 g of agarose powder, pour it into a conical flask, add 35 mL of buffer solution into the conical flask, heat it in a microwave oven until the agarose powder is completely melted, cool it to about 60˚C at room temperature, add 3 μL of EB solution, mix it evenly, pour the gel, and wait for the agarose gel to solidify and form.

Nucleic acid electrophoresis [19]: Place agarose gel blocks into an electrophoresis apparatus. The first empty sample is loaded with 4 μL of Marker III. Then, add 3 μL of 6 × Loading buffer to the sample in sequence, mix well, and add to the gel well. Set the voltage to 130 V; The current is set to 130 mA; The time is set to 20 min.

Gel cutting: after the completion of nucleic acid electrophoresis, put the agarose gel into the gel imaging system, turn on the UV light, and cut the complete gel block with a knife for the next step of DNA gel recovery.

2.3.4. Adhesive Recycling

Use a column DNA gel recovery kit (Shanghai Shenggong) for DNA gel recovery.

Measure DNA gel recovery concentration: Use a UV spectrophotometer to measure the DNA concentration of the DNA gel recovery solution, and store the

remaining solution in a refrigerator at 4˚C for future use.

2.3.5. Construction of Carrier

Construct a vector connection system (6 μL): pMD19T vector 0.5 μL, gel recovered DNA 2.5 μL, Solution I 3 μL. Store overnight at 16˚C in a dry thermostat.

2.3.6. Conversion

Add 50 μL of competent Escherichia coli DH5 α to a 6 μL plasmid system on a clean bench [20]-[22].

First, immerse the mixture in an ice bath for 20 minutes, then place it in a metal bath at 42˚C for 45 s of thermal stimulation, and finally ice bath again for 3 min.

Add 800 μL of SOC solution to the mixture on a super clean workbench and incubate for 1 h in a shaker with a speed of 200 rpm and a temperature of 37˚C. Then centrifuge at 4000 rpm for 1 min at room temperature.

When discarding the supernatant to about 100 μL on the ultra clean workbench, mix the remaining supernatant and precipitate evenly with a 100 mL pipette. Use a flame sterilized coating rod to apply the solution to antibiotic resistant LB plates and incubate at 37˚C for 10 - 12 h.

Observe the condition of the bacterial colonies, and proceed to the next step of bacterial selection when there are viable colonies to pick.

2.3.7. Bacterial Picking

On the ultra clean workbench, use sterilized toothpicks to pick up colonies and transfer them to 15 mL centrifuge tubes containing 4 mL of culture medium (Amp100 LB ratio: 1:1000) (6 in parallel). Incubate in a shaker at 37˚C and 200 rmp for 12 hours.

2.3.8. Plasmid Extraction (SDS Alkaline Lysis Method)

Take 1 mL of culture on a clean bench and centrifuge at 12,000 rpm for 30 seconds in a 1.5 mL EP tube at room temperature. Discard the supernatant, invert the EP tube onto toilet paper to remove the supernatant, add 200 μL of Solution I solution, vigorously shake to suspend the bacterial cells, add 400 μL of Solution II solution, invert the centrifuge tube 5 - 6 times, and lyse the bacterial solution [21]. At this time, the DNA denatures and the solution becomes clear, and there is a DNA drawing phenomenon when opening the tube mouth.

Add 300 μL of Solution III to the EP tube, invert 5 - 6 times, add 10 μL of chloroform to the EP tube and mix evenly. At room temperature, centrifuge at a speed of 13,000 rpm for 8 min.

Carefully transfer the supernatant into a new 1.5 mL EP tube, add 500 μL of isopropanol, shake vigorously and mix well. Place the EP tube in a low-temperature freezer at −20˚C for 10 minutes before taking it out. Centrifuge at a speed of 15,000 rpm and a temperature of 4˚C for 10 min.

Pour the supernatant into the waste liquid tank, add 1 mL of 75% ethanol to clean the precipitate, centrifuge at 12,000 rpm for 2 minutes, discard the alcohol, and dry with a 10 μL pipette tip.

Dry the precipitate at 65˚C for 2 min, add 20 μL of 1 × TE (pH 8.0, containing Rnase 20 μg/mL) to dissolve, and incubate at 37˚C for 30 min.

Electrophoresis: take 2 μL plasmid, add 2 μL 6 × Loding buffer, mix it on 1% agarose gel, and add it evenly into the gel hole. Setting procedure: voltage 120 V; Current 130 mA; After 20 min of electrophoresis, the bands can be used to construct the plasmid digestion system. After electrophoresis detection, store the plasmids that meet the standards at −20˚C.

2.3.9. Plasmid Digestion and PCR

Constructing a universal enzyme digestion system (20 μL): ddH_2O 16 μL, 10 × Buffer 2 μL, QuikCut EcoR I 1 μL, Place 1 μL of plasmid DNA in a constant temperature incubator at 37˚C for 1 h. Electrophoresis again, add Marker III to the first hole, then load samples in turn, and put them into the gel imaging system to observe the connection. Select samples larger than 3200 bp for detection. If no available samples are available, try plasmid PCR.

Construction of plasmid PCR system (20 μL): Taq 0.25 μL, 10 × buffer 2 μL, ITS2 forward and reverse primers each 0.5 μL, dNTP 2 μL, Plasmid 0.2 μL, ddH_2O 14.55 μL. Put it into a PCR amplification instrument; the reaction procedure is the same as that of total DNA PCR amplification, perform electrophoresis, observe the results, and if the 500 bp sample is met, the bacterial solution can be retained for detection.

2.3.10. Preservation and Testing of Bacterial Solution

Storage of bacterial solution: Take 0.6 mL of bacterial solution and 0.6 mL of 40% glycerol from a super clean bench, add them to a 1.5 mL EP tube, label the number, wrap them with sealing film, and store them in a −80˚C refrigerator for later use.

Submission for testing: Take 1 mL of bacterial solution and send it to General Bio (Anhui) Co., Ltd. for DNA sequencing.

3. Results and Analysis

3.1. Results of Genomic DNA Concentration in Angelica Sinensis

Genomic DNA was extracted from eight groups of Angelica sinensis samples using a plant DNA extraction kit, and their concentration and purity were measured. The results are shown in **Table 1**.

3.2. Results of ITS2 Sequence Amplification

Eight groups of Angelica sinensis samples were amplified by PCR and subjected to nucleic acid electrophoresis after adding A tail. The amplified bands were all around 500 bp (**Figures 1**-**4**). **Figure 1** shows the amplification results of ITS2 primer sequences for samples L1 and X1, **Figure 2** shows the amplification results of ITS2 primer sequences for samples M1, M2, and M3, **Figure 3** shows the amplification results of ITS2 primer sequences for samples M4 and M5, and **Figure 4** shows the amplification results of ITS2 primer sequences for sample M6.

Table 1. Results of DNA concentration and purity determination for 8 groups of Angelica sinensis samples.

sample	DNA concentration /(ng/μL)	A_{260}/A_{280}
L1	12.7	1.75
X1	15.1	2.09
M1	18.7	1.98
M2	12.7	1.82
M3	18.1	1.99
M4	15.1	1.86
M5	15.3	1.78
M6	17.8	2.18

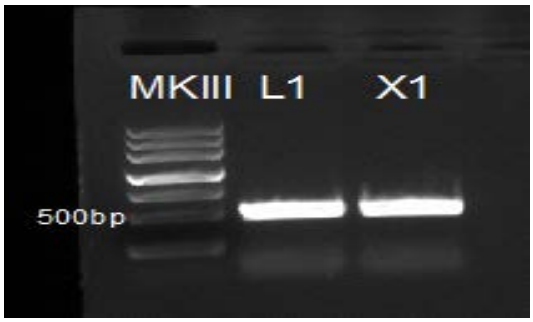

Figure 1. Nucleic acid electrophoresis results of Angelica sinensis samples L1 and X1.

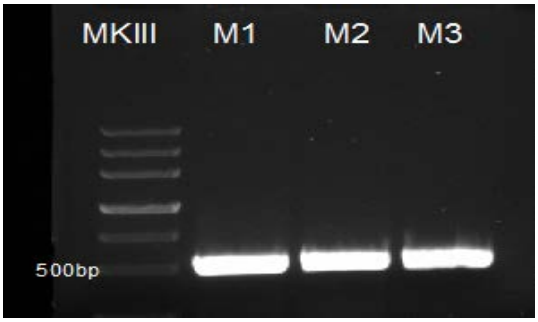

Figure 2. Nucleic acid electrophoresis results of Angelica sinensis samples M1, M2, and M3.

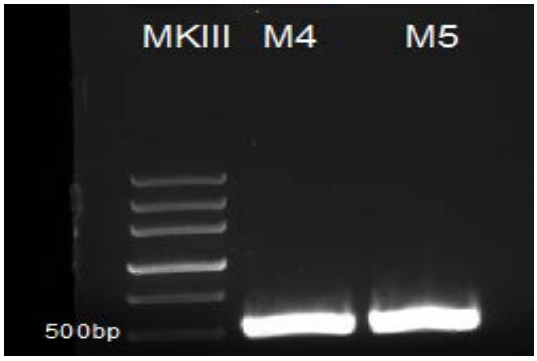

Figure 3. Nucleic acid electrophoresis results of Angelica sinensis samples M4 and M5.

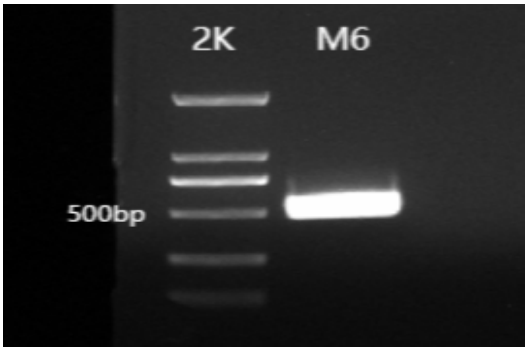

Figure 4. Nucleic acid electrophoresis results of Angelica sinensis samples M6.

3.3. Plasmid Enzyme Digestion and Plasmid PCR Results

Eight groups of Angelica sinensis samples were subjected to plasmid enzyme digestion, resulting in a band of approximately 3200 bp (**Figures 5-10**), while plasmid PCR was able to produce a band of approximately 500 bp, indicating successful cloning of the ITS sequence. The successfully cloned samples are X12 in **Figure 5** X13, L15, L16, M13 and M15 in **Figure 6**, M22 in **Figure 7** M23, M61, M63, M35 in **Figure 8**, M45 in **Figure 9** M46, M53 in **Figure 10** M54.

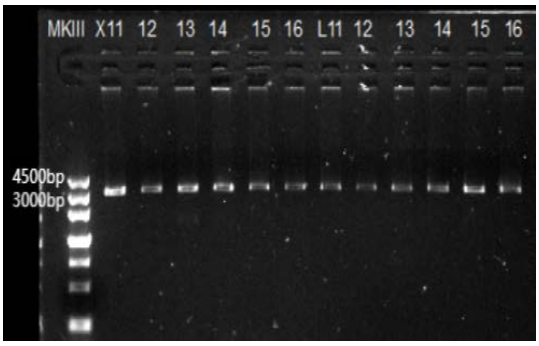

Figure 5. Plasmid enzyme digestion results of Angelica sinensis samples X1 and L1.

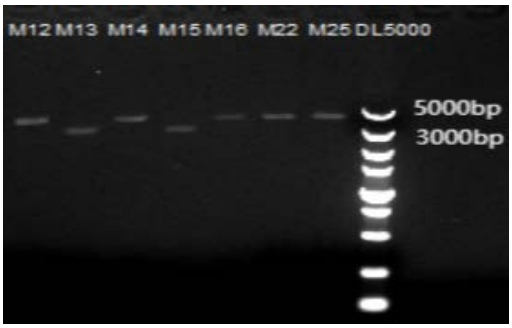

Figure 6. Plasmid enzyme digestion results of Angelica sinensis samples M1.

3.4. Comparison between Test Sequence Results

Using DNAMAN software, partial ITS2 fragments of the 15 groups of Angelica sinensis samples were compared between the same samples, and the results showed no differences between the same samples (see **Figures 11-17** for comparison).

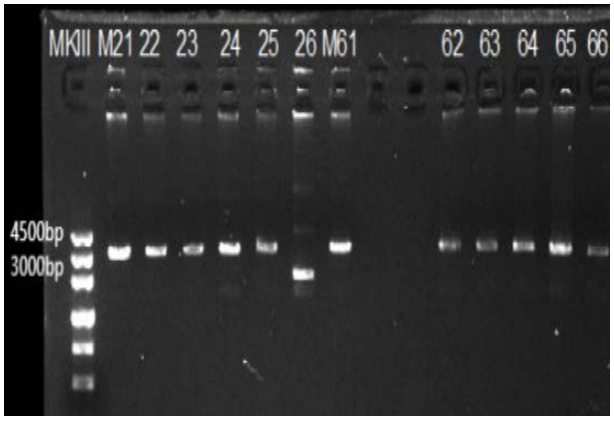

Figure 7. Plasmid enzyme digestion results of Angelica sinensis samples M2 and M6.

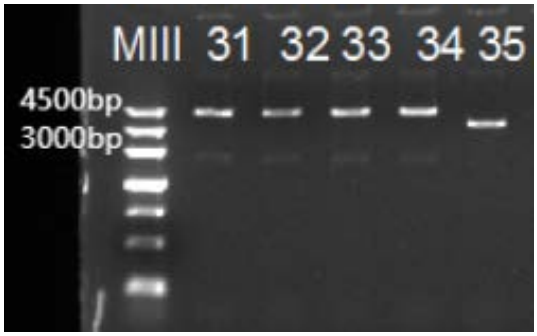

Figure 8. Plasmid enzyme digestion results of Angelica sinensis samples M3.

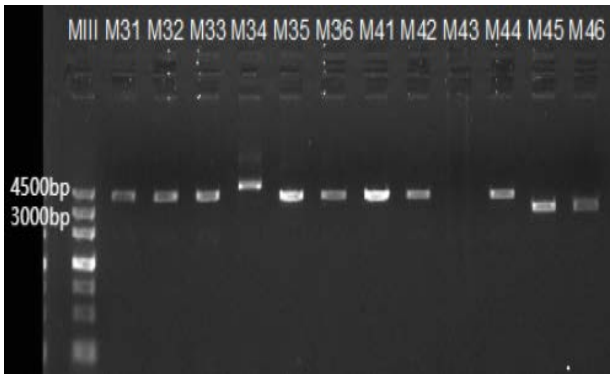

Figure 9. Plasmid enzyme digestion results of Angelica sinensis samples M4.

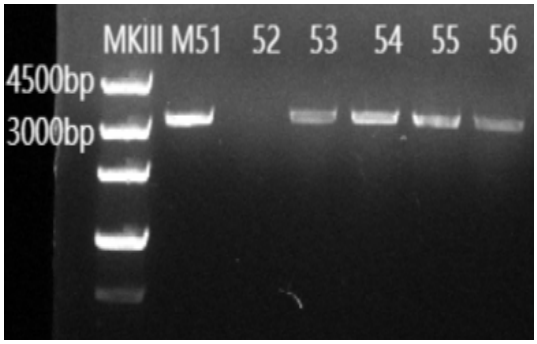

Figure 10. Plasmid enzyme digestion results of Angelica sinensis samples M5.

```
ITS2X12.txt    CGCATCATCTTTGCCCACAACCACTCACTCCTCGTGGAGC    40
ITS2X13.txt    CGCATCATCTTTGCCCACAACCACTCACTCCTCGTGGAGC    40
Consensus      cgcatcatctttgcccacaaccactcactcctcgtggagc

ITS2X12.txt    TGTACTGGTATGGGGGCGGAAATTGGCCTCCCGTGCCTTG    80
ITS2X13.txt    TGTACTGGTATGGGGGCGGAAATTGGCCTCCCGTGCCTTG    80
Consensus      tgtactggtatgggggcggaaattggcctcccgtgccttg

ITS2X12.txt    TTGTGCGGTTGGCGCAAAAGTGAGTCTCCGGCGACGGACG    120
ITS2X13.txt    TTGTGCGGTTGGCGCAAAAGTGAGTCTCCGGCGACGGACG    120
Consensus      ttgtgcggttggcgcaaaagtgagtctccggcgacggacg

ITS2X12.txt    TCGTGACATTGGTGGTTGTAAAATACCCTCATGTCTTGTC    160
ITS2X13.txt    TCGTGACATTGGTGGTTGTAAAATACCCTCATGTCTTGTC    160
Consensus      tcgtgacattggtggttgtaaaataccctcatgtcttgtc

ITS2X12.txt    GCGCGAATCCGCGTCATCTTAGTGAGCTCAAGGACCCTTA    200
ITS2X13.txt    GCGCGAATCCGCGTCATCTTAGTGAGCTCAAGGACCCTTA    200
Consensus      gcgcgaatccgcgtcatcttagtgagctcaaggacccttа

ITS2X12.txt    GGCGGCACACACTTTGTGCACTTCGAAT                228
ITS2X13.txt    GGCGGCACACACTTTGTGCACTTCGAAT                228
Consensus      ggcggcacacactttgtgcacttcgaat
```

Figure 11. Partial ITS2 comparison of Angelica sinensis samples X12 and X13.

```
ITS2L15.txt    TGTGCGCTTCGACTGTGACCCCAGGTCAGGCGGGACTACC    40
ITS2L16.txt    TGTGCGCTTCGACTGTGACCCCAGGTCAGGCGGGACTACC    40
Consensus      tgtgcgcttcgactgtgaccccaggtcaggcgggactacc

ITS2L15.txt    CGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAGAAAC    80
ITS2L16.txt    CGCTGAGTTTAAGCATATCAATAAGCGGAGGAAAAGAAAC    80
Consensus      cgctgagtttaagcatatcaataagcggaggaaaagaaac

ITS2L15.txt    TTACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAAC    120
ITS2L16.txt    TTACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAAC    120
Consensus      ttacaaggattcccctagtaacggcgagcgaaccgggaac

ITS2L15.txt    AGCCCAGCTTGAAAATTGGTCGGCTCTGCCGTCCGAATTG    160
ITS2L16.txt    AGCCCAGCTTGAAAATTGGTCGGCTCTGCCGTCCGAATTG    160
Consensus      agcccagcttgaaaattggtcggctctgccgtccgaattg

ITS2L15.txt    TAGTCTGGA                                   169
ITS2L16.txt    TAGTCTGGA                                   169
Consensus      tagtctgga
```

Figure 12. Partial ITS2 comparison of Angelica sinensis samples L15 and L16.

Further selection was made among all 15 groups of samples, and one group of samples was randomly selected from each of the eight types of samples, including X1 and L1, for comparison, as shown in **Figure 1**8. From the first row of base alignment, it can be seen that the eight groups of samples are roughly divided into three categories. The first category is X1 M2, M5, M6; The second category is M1 M3, M4; The third category is L1.

```
its2_m13.txt    CAGTCGAAGCGCACAGAGTACGTGCTGCCTAAGGGTCCTG    40
its2_m15.txt    CAGTCGAAGCGCACAGAGTACGTGCTGCCTAAGGGTCCTG    40
Consensus       cagtcgaagcgcacagagtacgtgctgcctaagggtcctg

its2_m13.txt    GAGCTCTCTAAGATGACAAGGATTCACGCGACAAGACAAG    80
its2_m15.txt    GAGCTCTCTAAGATGACAAGGATTCACGCGACAAGACAAG    80
Consensus       gagctctctaagatgacaaggattcacgcgacaagacaag

its2_m13.txt    AGGGTCTTTTACAACCACCGATGTCGCGACGTCCATCGCC    120
its2_m15.txt    AGGGTCTTTTACAACCACCGATGTCGCGACGTCCATCGCC    120
Consensus       agggtcttttacaaccaccgatgtcgcgacgtccatcgcc

its2_m13.txt    GGAGACTCATTTTTCCGCCAACCGCACGACAAGGTACGGG    160
its2_m15.txt    GGAGACTCATTTTTCCGCCAACCGCACGACAAGGTACGGG    160
Consensus       ggagactcatttttccgccaaccgcacgacaaggtacggg

its2_m13.txt    AGGCCAATTTCCGCCCCAAACCGGCACAACTTCTCAGGTG    200
its2_m15.txt    AGGCCAATTTCCGCCCCAAACCGGCACAACTTCTCAGGTG    200
Consensus       aggccaatttccgccccaaaccggcacaacttctcaggtg

its2_m13.txt    TGACTGGTTTGTGGGCAATACAATGC                  226
its2_m15.txt    TGACTGGTTTGTGGGCAATACAATGC                  226
Consensus       tgactggtttgtgggcaatacaatgc
```

Figure 13. Partial ITS2 comparison of Angelica sinensis samples M13 and M15

```
ITS2M22.txt     CGCATCATCTTTGCCCACAACCACTCACTCCTCGTGGAGC    40
ITS2M23.txt     CGCATCATCTTTGCCCACAACCACTCACTCCTCGTGGAGC    40
Consensus       cgcatcatctttgcccacaaccactcactcctcgtggagc

ITS2M22.txt     TGTACTGGTATGGGGGCGGAAATTGGCCTCCCGTGCCTTG    80
ITS2M23.txt     TGTACTGGTATGGGGGCGGAAATTGGCCTCCCGTGCCTTG    80
Consensus       tgtactggtatgggggcggaaattggcctcccgtgccttg

ITS2M22.txt     TTGTGCGGTTGGCGCAAAAGTGAGTCTCCGGCGACGGACG    120
ITS2M23.txt     TTGTGCGGTTGGCGCAAAAGTGAGTCTCCGGCGACGGACG    120
Consensus       ttgtgcggttggcgcaaaagtgagtctccggcgacggacg

ITS2M22.txt     TCGTGACATTGGTGGTTGTAAAATACCCTCATGTCTTGTC    160
ITS2M23.txt     TCGTGACATTGGTGGTTGTAAAATACCCTCATGTCTTGTC    160
Consensus       tcgtgacattggtggttgtaaaataccctcatgtcttgtc

ITS2M22.txt     GCGCGAATCCGCGTCATCTTAGTGAGCTCAAGGACCCTTA    200
ITS2M23.txt     GCGCGAATCCGCGTCATCTTAGTGAGCTCAAGGACCCTTA    200
Consensus       gcgcgaatccgcgtcatcttagtgagctcaaggacccctta

ITS2M22.txt     GGCGGCACACACTTTGTGCACTTCGAAT                228
ITS2M23.txt     GGCGGCACACACTTTGTGCACTTCGAAT                228
Consensus       ggcggcacacactttgtgcacttcgaat
```

Figure 14. Partial ITS2 comparison of Angelica sinensis samples M22 and M23.

```
its2_m45.txt    CAGTCGAAGCGCACAGAGTACGTGCTGCCTAAGGGTCCTG    40
its2_m46.txt    CAGTCGAAGCGCACAGAGTACGTGCTGCCTAAGGGTCCTG    40
Consensus       cagtcgaagcgcacagagtacgtgctgcctaagggtcctg

its2_m45.txt    GAGCTCTCTAAGATGACAAGGATTCACGCGACAAGACAAG    80
its2_m46.txt    GAGCTCTCTAAGATGACAAGGATTCACGCGACAAGACAAG    80
Consensus       gagctctctaagatgacaaggattcacgcgacaagacaag

its2_m45.txt    AGGGTCTTTTACAACCACCGATGTCGCGACGTCCATCGCC    120
its2_m46.txt    AGGGTCTTTTACAACCACCGATGTCGCGACGTCCATCGCC    120
Consensus       agggtcttttacaaccaccgatgtcgcgacgtccatcgcc

its2_m45.txt    GGAGACTCATTTTTCCGCCAACCGCACGACAAGGTACGGG    160
its2_m46.txt    GGAGACTCATTTTTCCGCCAACCGCACGACAAGGTACGGG    160
Consensus       ggagactcatttttccgccaaccgcacgacaaggtacggg

its2_m45.txt    AGGCCAATTTCCGCCCCAAACCGGCACAACTTCTCAGGTG    200
its2_m46.txt    AGGCCAATTTCCGCCCCAAACCGGCACAACTTCTCAGGTG    200
Consensus       aggccaatttccgccccaaaccggcacaacttctcaggtg

its2_m45.txt    TGACTGGTTTGTGGGCAATACAATGC                  226
its2_m46.txt    TGACTGGTTTGTGGGCAATACAATGC                  226
Consensus       tgactggtttgtgggcaatacaatgc
```

Figure 15. Partial ITS2 comparison of Angelica sinensis samples M45 and M46.

```
ITS2M53.txt     CGCATCATCTTTGCCCACAACCACTCACTCCTCGTGGAGC    40
ITS2M54.txt     CGCATCATCTTTGCCCACAACCACTCACTCCTCGTGGAGC    40
Consensus       cgcatcatctttgcccacaaccactcactcctcgtggagc

ITS2M53.txt     TGTACTGGTATGGGGGCGGAAATTGGCCTCCCGTGCCTTG    80
ITS2M54.txt     TGTACTGGTATGGGGGCGGAAATTGGCCTCCCGTGCCTTG    80
Consensus       tgtactggtatgggggcggaaattggcctcccgtgccttg

ITS2M53.txt     TTGTGCGGTTGGCGCAAAAGTGAGTCTCCGGCGACGGACG    120
ITS2M54.txt     TTGTGCGGTTGGCGCAAAAGTGAGTCTCCGGCGACGGACG    120
Consensus       ttgtgcggttggcgcaaaagtgagtctccggcgacggacg

ITS2M53.txt     TCGTGACATTGGTGGTTGTAAAATACCCTCATGTCTTGTC    160
ITS2M54.txt     TCGTGACATTGGTGGTTGTAAAATACCCTCATGTCTTGTC    160
Consensus       tcgtgacattggtggttgtaaaataccctcatgtcttgtc

ITS2M53.txt     GCGCGAATCCGCGTCATCTTAGTGAGCTCAAGGACCCTTA    200
ITS2M54.txt     GCGCGAATCCGCGTCATCTTAGTGAGCTCAAGGACCCTTA    200
Consensus       gcgcgaatccgcgtcatcttagtgagctcaaggacccttа

ITS2M53.txt     GGCGGCACACACTTTGTGCACTTCGAAT                228
ITS2M54.txt     GGCGGCACACACTTTGTGCACTTCGAAT                228
Consensus       ggcggcacacactttgtgcacttcgaat
```

Figure 16. Partial ITS2 comparison of Angelica sinensis samples M53 and M54.

```
ITS2M61.txt    CGCATCATCTTTGCCCACAACCACTCACTCCTCGTGGAGC    40
ITS2M63.txt    CGCATCATCTTTGCCCACAACCACTCACTCCTCGTGGAGC    40
Consensus      cgcatcatctttgcccacaaccactcactcctcgtggagc

ITS2M61.txt    TGTACTGGTATGGGGGCGGAAATTGGCCTCCCGTGCCTTG    80
ITS2M63.txt    TGTACTGGTATGGGGGCGGAAATTGGCCTCCCGTGCCTTG    80
Consensus      tgtactggtatgggggcggaaattggcctcccgtgccttg

ITS2M61.txt    TTGTGCGGTTGGCGCAAAAGTGAGTCTCCGGCGACGGACG    120
ITS2M63.txt    TTGTGCGGTTGGCGCAAAAGTGAGTCTCCGGCGACGGACG    120
Consensus      ttgtgcggttggcgcaaaagtgagtctccggcgacggacg

ITS2M61.txt    TCGTGACATTGGTGGTTGTAAAATACCCTCATGTCTTGTC    160
ITS2M63.txt    TCGTGACATTGGTGGTTGTAAAATACCCTCATGTCTTGTC    160
Consensus      tcgtgacattggtggttgtaaaataccctcatgtcttgtc

ITS2M61.txt    GCGCGAATCCGCGTCATCTTAGTGAGCTCAAGGACCCTTA    200
ITS2M63.txt    GCGCGAATCCGCGTCATCTTAGTGAGCTCAAGGACCCTTA    200
Consensus      gcgcgaatccgcgtcatcttagtgagctcaaggacccttа

ITS2M61.txt    GGCGGCACACACTTTGTGCACTTCGAAT                228
ITS2M63.txt    GGCGGCACACACTTTGTGCACTTCGAAT                228
Consensus      ggcggcacacactttgtgcacttcgaat
```

Figure 17. Partial ITS2 comparison of Angelica sinensis samples M61 and M63.

3.5. Comparison between Sequencing Results and Database

From the above results, it can be seen that the eight groups of Angelica samples can be divided into three categories. Randomly select a set of available samples from each category for gene database comparison. The results show that samples X1, M2, M5, and M6 have a similarity of 98.99% with the ITS2 gene of Angelica sinensis in the database (GenBank number: KC295071), indicating that these four samples are Angelica sinensis; The similarity between samples M1, M3, M4 and the ITS2 gene of Angelica dahurica in the database (GenBank number: OK668234) is 95.56%, indicating that these three samples are Angelica dahurica; The similarity between sample L1 and the ITS2 gene of Shifengfeng in the database (GenBank number: KC295082) is 98.99%, indicating that this sample is Kitagawia terebinthacea.

4. Conclusion

This project aims to study the method of identifying the authenticity of Angelica sinensis based on genes. By extracting genomic DNA from 8 groups of Angelica sinensis samples, conducting PCR amplification, TA cloning, and DNA sequencing, the sequencing results were compared with the gene library to identify the authenticity of Angelica sinensis samples. The results showed that the four samples were Angelica sinensis, with a similarity of 98.99% to the ITS2 gene of Angelica sinensis in the database (Gen Bank number: KC295071); Three samples are

Figure 18. Partial ITS2 comparison of 8 groups Angelica sinensis samples.

Angelica dahurica, with a similarity of 95.56% to the ITS2 gene of Angelica dahurica in the database (Gen Bank number: OK668234); One sample is Kitagawia terebinthacea, with a similarity of 98.99% to the ITS2 gene of Shifangfeng in the database (Gen Bank number: KC295082). This project is based on the ITS2 sequence identification method for the authenticity of Angelica sinensis, which can accurately identify the authenticity of Angelica sinensis samples and provide reference for Angelica sinensis identification.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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