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Application of Molecular Biology Technology in Identification of Modern Mongolian Medicine

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Abstract The application of molecular biology technology in the identification and quality control of Mongolian medicine is increasing gradually, and it provides a new method for identifying fake and inferior products and confused products of Mongolian medicine. In this paper, the application and prospect of molecular biology technology (such as DNA barcoding and PCR molecular identification technique) in the identification of crude Mongolian medicine were reviewed.

Key words Molecular biology, Mongolian medicine, PCR technology, DNA barcoding technology

1 Introduction

Mongolian medicine is the main part of Chinese traditional medicine and the precious cultural heritage of the Chinese nation, and has played an indelible role in the practice of preventing and curing diseases. Mongolian drugs are an extremely important part of Mongolian medicine. Through the accumulation of long-term practical experience, Mongolian ancestors had a certain understanding of food for relieving or treating pain, and then consciously applied them to alleviate diseases, thus forming the knowledge of Mongolian drugs and Mongolian medicine[1]. There are many varieties of Mongolian drugs, and they are mainly widely distributed in various regions of China. They are collected from plants, animals and minerals. 2 000 kinds of medicinal materials are recorded in the ancient books on Chinese materia medica in Mongolian medicine[2]. *Book on Inerrant Mongolian Medicine* is the first book on Chinese materia medica to record the diagrams of Mongolian medicinal materials, contains 663 kinds of diagrams, and has made great contributions to the identification of medicinal materials. In recent years, the country has also strengthened the development path of modern industrialization of Mongolian medicine, so it is urgent to formulate and improve the quality standards of Mongolian medicinal materials and preparations, and conduct a lot of research on the basic source of Mongolian medicinal materials and resource investigation. However, due to the confusion of geographical environment and varieties of Mongolian medicinal materials, the basic source is complicated[3]. In quality control, the same medicinal material comes from different origins or different natural wild and artificial cultivation and growth environment, so there is a difference in quality and efficacy of Mongolian medicinal materials, which brings great difficulties for the identification method of Mongolian medicinal materials, but now it is difficult to meet the requirements of identification only by relying on traditional identification methods. Besides, the phenomenon of foreign bodies of the same genus or the same name in Mongolian medicine is common, and the factors such as fake and inferior products and confused products[4-5] have a significant impact on the research on the effective active ingredients and pharmacological effects of medicinal materials, as well as the clinical efficacy, production and promotion of medicinal materials, which further limits the industrialization and development space of Mongolian medicine.

With the development of life science and technology, especially the rapid development of microbiology, more and more researchers apply molecular biotechnology of emerging and comprehensive disciplines to the identification of Mongolian medicinal materials, gene cloning and function research, variety improvement of Mongolian medicine, improvement of quality of medicinal materials, synthesis of active ingredients and regulation in Mongolian medicine[6-8]. In addition, DNA sequencing technology and polymerase chain reaction (PCR) method were added to the identification of medicinal materials in the *Chinese Pharmacopoeia* (2020)[9], indicating that this technology has wide application and strong practicability in the identification of crude drugs. In this paper, the application of molecular biotechnology in the identification of modern Mongolian medicine is mainly introduced, with emphasis on PCR technology and DNA barcode technology.

2 Traditional identification

The earliest Mongolian medicine practitioners identified the appearance of medicinal materials by simple methods of looking, smelling, tasting and touching them, including size, shape, surface, section, texture, odor, *etc.*[10], and they are practical and empirical methods. The traditional identification terms of Mongolian medicinal materials are not only one of the characteristics of Mongolian medicine[11], but also the accumulation of experience of Mongolian medicine practitioners over thousands of years and the generalization and induction of classic works. Domestic and

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foreign researchers use electronic smell, taste, and visual bionic technology to identify the authenticity of medicinal materials[12-15]. At present, the bionic identification technology has not been used in the authenticity identification and quality control of Mongolian medicinal materials. Under the condition of abundant human, material and financial resources, the bionic identification method of Mongolian medicinal materials can be studied, which not only applies modern science and technology to study the identification of Mongolian medicine, but also inherits and carries forward the valuable characteristic identification terminology of Mongolian medicine, having a good research prospect.

3 Modern identification technology

With the development of science and technology and the deepening of human research on medicinal plants, the identification and discernment of medicinal materials have become a hot spot in quality detection, resource investigation and base source research. After processing, the shape and microscopic characteristics of most medicinal materials will change slightly, so that it is difficult to identify fake and bad products, confusing products, *etc.*, and there are even cases of deliberate counterfeiting in the medicinal market. Researchers have widely used microscopic identification of traits, identification technology of biological effects (identifying the advantages and disadvantages of medicinal materials based on drug efficacy) and chemical identification methods (including mass spectrometry, spectroscopy, and chromatography) to identify medicinal materials[16-17]. However, it is found that this technology requires advanced equipment and professional identification talents, and it is not suitable for the identification of animal medicinal materials. In recent years, rapid and accurate identification of species using modern molecular identification methods has also been applied to the identification of Mongolian medicinal materials and compound preparations.

3.1 DNA barcoding identification technology DNA barcoding refers to a novel identification technique that uses a standard DNA sequence as a marker to identify biological species rapidly and accurately. Canadian zoologist Paul Hebert first proposed the coding technology of using bar codes in the identification of biological species[18-19], which is similar to convenience stores that scan bar codes to identify thousands of different items. At present, it has a wide application prospect in various fields. It will not only further develop the traditional taxonomic research, but also ccelerate the identification work, so as to effectively utilize Mongolian medicinal materials. There are few reports on the identification of Mongolian medicinal materials and their compounds by molecular biotechnology. For instance, Chen Shilin *et al.*[20] showed that a species information database, DNA barcode sequence and primer database, database management system and species identification system should be established for the application of this DNA barcoding technology. Besides, Zhang Chuanling *et al.*[21-22] reported that when DNA barcoding technology is applied to the identification of immature Mongolian medicine, the research on medicinal plant resources and maternal-herbal research of Mongolian medicine had been thoroughly carried out, and the screening and identification of DNA bar codes should be carried out. In 2010, it was reported that the gene sequence clones of four Mongolian medicinal materials had been included in the gen bank, including *Ligularia przewalskii*, *Pleuro-spermum camtschaticum*, *Syringa oblata* and *L. rota-tum*. Meanwhile, it was also reported that five Mongolian medicinal plants of the genus *Vicia* had obtained the international ITS sequences, including *Vicia pseudo-orobus* Fisch. & C. A. Mey. (JQ309788), *V. amurensis* Oett. (JQ309789), *V. multicaulis* Ledeb. (JQ309791), *V. japonica* Kom. (JQ309792) and *V. ramuliflora* (Maxim.) Ohwi (JQ309793). Morerover, the ITS sequences of 6 Mongolian medicinal plants of the genus *Clematis* were submitted to the NCBI database[23-24]. ITS sequences can be used to identify four "Digeda" medicinal plants of Gentianaceae and establish a method for the identification of flatstem milkvetch seeds[25-26]. At the same time, it is found that the identification rate of ITS2 sequences from the gene bank is very high for plants, and also high for animals. Therefore, it is proposed that ITS2 is the standard DNA bar code of medicinal plants, also showing that ITS2 is the filling gene sequence of the special bar code COI for animals. Some scholars used the DNA bar code of ITS2 gene sequences to establish a method for identification of Mongolian medicinal plants *Scutellaria scordifolia*, *Dracocephalum moldavica* L., *Glycyrrhiza uralensis* Fisch., and Aconiti Kusnezoffii Folium, and the results show that ITS2 sequences could identify them accurately and quickly, and provide an effective basis for the authenticity identification and quality control of this kind of Mongolian medicinal materials[27-30].

3.2 PCR molecular identification technique In 1985, American Karray proposed polymerase chain reaction (PCR) technology. It is developed by the American Cetus Company, as the second generation of DNA molecular markers. The principle is similar to the DNA replication process, in particular relying on oligonucleotide primers complementary to both ends of the target sequence. Based on three basic reaction steps, the denaturation-annealing (renaturation) -extension process of PCR is formed. Based on PCR technology, some techniques are also gradually applied in the field of Mongolian medicine identification. For example, techniques RAPD (random amplification polymorphism DNA), ISSR-PCR (inter-simple sequence repeat), and SSR (simple sequence repeat) are mainly used for the identification of the authenticity and quality of medicinal materials, and AFLP (amplified fragment length polymorphism) technology is mainly used in the genetic diversity and identification of medicinal plants[3-31]. Some scholars reported that techniques RAPD and ISSR were used to analyze the genetic diversity of Mongolian medicine "Agei" and the obvious differences between different places. The results show that the genetic diversity was mainly derived from the population, and there was also gene communication between the populations[32-34]. PCR technology is simple, fast and accurate in the identification of medicinal plants, and has been gradually applied to the molecular identification of Mongolian medicine. However, there are many factors that can influence the experiment of this technology, and strict requirements are required for laboratory management and control to obtain experimental results with good repeatability and high accuracy.

4 Outlooks

Molecular biology technology is in the initial stage of Mongolian medicine research, facing many problems, and there are practical difficulties in screening and identification. However, compared with traditional identification methods, it has high accuracy and better repetition, is rapid and not limited by plant morphology, and requires less detection amount, so it can reduce the waste of medicinal materials. It is more practical and feasible to develop the identification technology of Mongolian medicinal plants, and provides a novel research method for the quality control and modernization of Mongolian medicine.

With the development of Mongolian medicine research, molecular biology will be widely used in Mongolian medicine research. Combining with modern advanced science and technology and biology, it will promote the development of Mongolian medicine research and Mongolian medicine science. In short, it is necessary to inherit and carry forward the unique features of Mongolian medicine, and learn from the theories of modern molecular biology, pharmacology, chemistry and other disciplines to speed up the modernization research of Mongolian medicine.

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