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DNA BARCODING FOR IDENTIFICATION OF CRUDE DRUGS

***Namrata Sonawane, Swasti Jain**

Smt.Sharadchandrika Suresh Patil College of Pharmacy Chopda, Tal chopda Dist Jalgaon 425107 India.

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***Corresponding Author: Namrata Sonawane**

Smt.Sharadchandrika Suresh Patil College of Pharmacy Chopda, Tal chopda Dist Jalgaon 425107 India.

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ABSTRACT

The rapid growth of the herbal and ayurvedic drug industry has necessitated increasing interest in dependable identification methods that guarantee authenticity, purity, and safety of the crude drug materials. Traditional approaches involving morphology, microscopy, or phytochemical profiling very often prove to be inadequate in cases of powdered, processed, or intentionally adulterated samples with visually similar materials. DNA barcoding has emerged as an exact, reproducible, and universally applicable method at a molecular level for species authentication. The method of DNA barcoding, through the analysis of short, standardized genetic loci such as ITS2, rbcL, and psbA-trnH, can correctly identify even closely related taxa and reveal intentional or unintentional substitution in commercial herbal raw materials. The review consolidates major advances in DNA barcoding applications for crude drug verification, summarizes successful case studies for detection of adulteration, evaluates marker performances in complex matrices, and also shows integrated workflows combining barcoding with chemo profiling for enhanced quality assurance. The status of current gaps in research, including limited availability of reference sequences, challenges posed by degraded DNA, and a lack of sufficient validation for polyherbal formulations, is also presented. Addressing these gaps is bound to establish DNA barcoding as a routine component of regulatory quality-control frameworks and ensure global confidence in herbal medicine supply chains.

KEYWORDS: DNA barcoding, herbal drugs, adulteration, herbal, ayurvedic.

INTRODUCTION

The increasing preference for natural therapies, expanding research into plant-based

treatments, and growing awareness of wellness all make the world more eager for herbal medicines and Ayurvedic raw materials. Certainly, with growing demand, the need to have reliable methods for identification that guarantee authenticity and safety becomes increasingly critical.

Adulteration and substitution of crude herbal drugs are critical quality-control challenges that substantially compromise therapeutic efficacy, patient safety, and overall market credibility. Due to the replacement or admixture with incorrect species of botanical ingredients expected pharmacological activity declines, leading to inconsistent or ineffective clinical outcomes. Contaminants or toxic substitutes may introduce unexpected adverse effects, placing patients at significant risk.

DNA barcoding has emerged as a powerful and contemporary tool for authentication of herbal raw materials. Analysis of the short, species-specific DNA regions such as rbcL, matK, or ITS2 allows verification of the botanical origin even when the material has been dried, pulverized, or otherwise processed beyond recognition by gross morphological and microscopic features. Since physical degradation often leaves the genetic signature intact, DNA barcoding provides a degree of accuracy and reliability not possible from the traditional morphological and microscopic examination in powdered drugs.[1]

Traditional morphological and microscopic identification only works when the herbal materials retain visible structures such as leaves, roots, trichomes, vessels, or cell patterns. However, in powdered, dried, or processed herbal drugs, these diagnostic characters are destroyed and, as such, species identification becomes unreliable or impossible.

The aim of this review is to assess the progress made so far in the field of DNA barcoding in relation to the identification of botanicals. In the current paper, we review the genomic regions selected as possible barcodes for medicinal plants and the emerging new methods for rapid generation of barcodes. We also discuss the challenges of barcoding and what databases are available to retrieve barcodes of medicinal plants, their substitutes and adulterants.

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replacement or admixture with incorrect species of botanical ingredients expected pharmacological activity declines, leading to inconsistent or ineffective clinical outcomes. Contaminants or toxic substitutes may introduce unexpected adverse effects, placing patients at significant risk.

Principle of DNA Barcoding

DNA barcoding is a molecular approach for which a small, standardized segment of DNA is used to identify a species with high accuracy. Even fragmented or processed herbal materials can be reliably authenticated by comparing the amplified sequence to reference databases. This overcomes the limitations of morphological and chemical analyses and represents a rapid, objective method for detecting adulteration in crude herbal drugs.[2]

All the DNA barcodes proposed for plants comprise a few loci which are well-established and provide reliable species-level resolution.

RBCL, a chloroplast gene, is considered to have universal amplification and wide taxonomic coverage, though it provides only moderate

discrimination. matK is another chloroplast gene that is more variable and hence provides strong species-level resolution. Thirdly, the nuclear ribosomal ITS/ITS2 region is highly informative because of its rapid evolution and hence it finds useful application to closely related species. The chloroplast intergenic spacer psbA–trnH is also widely used because it is highly variable and easy to amplify. Overall, all these loci provide a robust multilocus framework for the authentication of crude herbal drugs.

DNA barcoding could particularly offer advantages regarding authentication of herbal drugs, since it allows species identification even in dried, powdered, or highly fragmented biological materials in which morphological features are lost. Failures in PCR amplification due to degraded DNA and incomplete or poorly curated reference databases, however, may impede correct species matching and thus in general reduce reliability.[4]

Future developments

Although the traditional DNA barcoding techniques remain an effective DNA method for identification of medicinal plants, the more advanced and newly developed high throughput sequencing, specifically next-generation sequencing (NGS) technologies[5], could be adopted and potentially revolutionize the process. Even though DNA barcoding usually targets short regions of DNA molecule within the genome and does not require full genome-scale data, the potential of using NGS to simultaneously

Challenges and limitations of barcoding

The isolation of pure, high molecular weight DNA is critical for the successful application of molecular methods. This can be quite a challenge since in processed medicinal plant material the DNA is often highly degraded or the plant material contains high amounts of polysaccharides, polyphenols and other secondary metabolites, such as, alkaloids and flavonoids. Various commercial kits and modified traditional methods are available to yield in good quality DNA from raw and powdered medicinal.

Availability of data

It is desirable to have access to a single barcode library for all medicinal material used (including fungal and animal species). Currently, however several barcode libraries are freely accessible (see also review by Taylor and Harris [6])

Comparison of DNA Mini-Barcoding and DNA Meta-Barcoding: Advantages and Disadvantages

Meta-barcoding is a technique that uses universal PCR primers to simultaneously amplify multiple DNA barcodes, identifying many species in single environmental samples . This is in contrast to DNA mini-barcoding, in which a single species is sequenced using PCR. In meta-barcoding studies on CPMs, ITS2 or other plant barcodes are frequently chosen because these sequences can produce high identification efficiency among many species. As sequencing technologies have developed, next-generation sequencing (NGS) has been widely used for NHP quality control. Most CPM prescriptions are very complex; hence, universal primer pairs for DNA barcode

amplification may not be feasible for the identification of herbal products using Sanger sequencing. Identification methods combined with NGS can identify species from multiple taxa. For example, Cheng et al., inspired by Taberlet et al., used ITS2 and trnL (P-loop) as biomarkers to analyze nine commercial Liuwei Dihuang Wan specimens in three batches using high-throughput sequencing (HTS). Different manufacturer samples contained different contaminations, and these contaminations may have occurred during the manufacturing process. Moreover, DNA from unprocessed Rehmannia glutinosa was successfully amplified, whereas DNA from processed R. glutinosa was not. This finding indicates the different detection efficiencies of unprocessed and processed herbal materials. Coghlan et al. analyzed CPMs using HTS and found unknown plant or animal sequences in 50% of samples; unexpectedly, the endangered snow leopard was detected[7]

Future Expectations for DNA Mini-Barcoding

Recent media reports and scientific studies highlight how widespread adulterations and ingredient substitution in NHP have become and underscore the threat to consumer safety.

DNA mini-barcoding can overcome NHP identification difficulties caused by DNA degradation after extensive processing. While this method will never supplant typical DNA barcoding in identifying

specimens, it can significantly improve the efficiency and accuracy of sample analysis by increasing the PCR amplification rate. New rapid detection methods based on mini-barcoding are expected to be established, particularly for processed products.

In general, mini-barcodes are species-specific, allowing their utility in analyzing complex samples. DNA mini-barcodes represent an

improved approach to carefully identify plant products and protect public health. This significant tool should be further developed and applied to the quality control of NHP[8]

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