DNA Barcoding to Identify Species Found in Traditional Herbal Medicines

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Background

New Zealand is a signatory of the international treaty known as CITES. CITES is the Convention on International Trade in Endangered Species (Figure 1). It regulates international trade in nearly 40,000 plant and animal species for the protection and preservation of their population levels.

Figure 1: Countries that are signatories of CITES

Current shortcomings

CITES parties are obliged to enforce the trade restrictions in the relevant species. Undertaking this enforcement is difficult for highly processed samples, such as traditional medicines. Typical visual species identification is ineffective; therefore, alternative methods are required.

Methods

A DNA barcoding workflow is being pursued, where short marker regions are sequenced and compared to known references.

Phase 1

An assessment of three DNA extraction kits in a diverse range of mock 'traditional medicine' samples.

✓ Complete

Phase 2

Genetic marker regions and PCR primers are chosen. PCR conditions are optimised using mock samples.

✓ Complete

Phase 3

The mock samples are sequenced on the Illumina platform and bioinformatic analysis is carried out.

In Progress

Phase 4

The completed workflow is applied to around 100 real traditional medicine samples.

Pending

Results

The Qiagen Food kit and Promega Dneasykits outperformed the Qiagen Plant Pro kit in terms of DNA yield and subsequent PCR success. Kit 2 is preferred to kit 1 due to lower toxicity of reagents. DNA was obtained even from highly processed inputs.

Figure 2 displays the amplification success of all five marker regions. The displayed figures are from one sample type only.

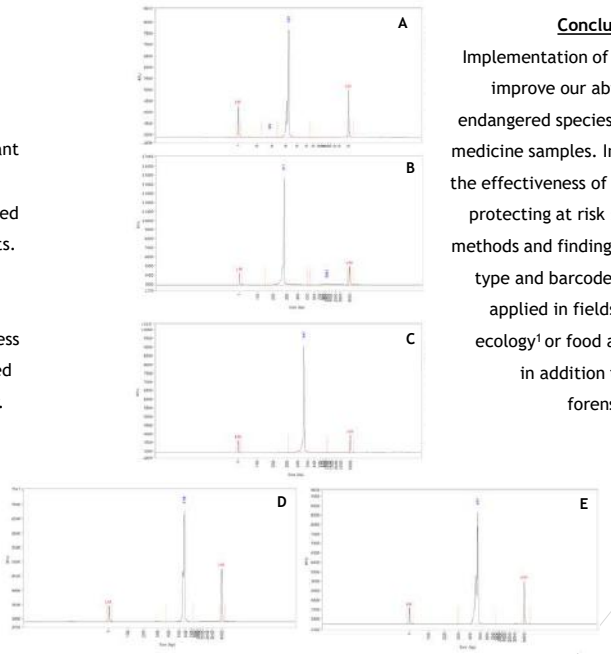

Figure 2: Capillary electrophoresis of the chosen five marker regions PCR products. A: the 16S rRNA amplicon at 323bp. B: the cytochrome B amplicon at 277bp. C: the cytochrome oxidase subunit II amplicon at 457bp. D: the ITS2 amplicon at 578bp. E: the rbcL amplicon at 471bp.

Conclusions

Implementation of this research will improve our ability to detect endangered species DNA in processed medicine samples. In turn, this will aid the effectiveness of the CITES treaty in protecting at risk species. Further, methods and findings regarding sample type and barcode regions may be applied in fields as diverse as ecology[1] or food authentication[2], in addition to wildlife forensics.

References

1. Moore, Tiara, Camille Gaynus, Phillip S. Levin, and Rachel Meyer. "The Intersection of Forensic Techniques with Ecological Issues." In *Wildlife Biodiversity Conservation*, pp. 147-161. Springer, Cham, 2021. https://doi.org/10.1007/978-3-030-64682-0_7

2. Saadat, Saeida, Hardi Pandya, Aayush Dey, and Deepak Rawtani. "Food forensics: Techniques for authenticity determination of food products." *Forensic Science International* (2022): 111243. https://doi.org/10.1016/j.forsciint.2022.111243