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DNA-based methods for the authentication of medicinal plant material

Background

Newsletter readers may be aware of the recent controversy sparked off by claims that a majority of the North American herbal products tested by DNA barcoding show evidence of adulteration (Newmaster *et al*., 2013). There has been an extensive rebuttal of these claims (Gafner *et al*., 2013), which may have left readers wondering what to think about DNA authentication technology. Is it a new, multi-purpose power-tool in the herbal medicine authentication toolbox, or have DNA methods been over-hyped?

Our research group has been working for a number of years to develop DNA authentication tests for medicinal plant material, for use in industry and by the regulatory authorities. Compared to existing botanical and chemical assays, DNA-based methods have a number of advantages. DNA tests directly indicate genetic identity, and are not affected by the tissue, age or growth conditions of the plant, nor the harvesting and processing of the plant material. DNA methods are highly *sensitive* (due to signal amplification by the polymerase chain reaction - PCR) and *specific* (due to the base-pairing of the DNA double helix). Therefore, our DNA tests are able to directly authenticate a plant species using a pinch of material from, say, a capsule of powdered plant extract. DNA-based methods are also very fast compared to chemical methods, and less expensive.

What does the future hold for DNA testing of herbal medicines?

These advantages do not mean that DNA will replace chemical testing in the future. We view DNA and chemical tests as complementary. DNA tests directly determine genetic identity, but only infer chemical composition. Chemical tests do the opposite – they are only an indirect indication of species identity, but directly measure chemical composition (including the levels of pharmaceutically active compounds). We have therefore adopted a nuanced approach to DNA testing. We see two key areas where DNA testing has major benefits and will become routine, and others where it is less appropriate:

1. *The use of DNA barcodes as a quality standard for species identification*.

   The international Barcode of Life initiative aims to catalogue a unique DNA "barcode" sequence for every known species on the planet. One of the objectives is that species identification for applied purposes (such as herbal medicine authentication) will no longer require the direct input of an expert taxonomist. It is entirely feasible that DNA barcode sequences could be introduced into the herbal product pharmacopoeia monographs as a quality standard for confirmation of species identity. This

would then require producers to DNA barcode their source materials and match them with the standard DNA barcode sequences to demonstrate compliance with the monograph.

2. *The use of simple PCR based tests for rapid, high throughput screening of source plant material*. This could be used for routine in-house testing of source materials, and would be particularly valuable for wild harvested material, where misidentification as well as deliberate adulteration can be an issue. The use of quantitative PCR methods to ensure that contamination of source material is below the 2% tolerance level is a possible extension of this approach.

How easy would it be for a company to adopt these DNA testing methods?

1. DNA barcoding

DNA barcoding, as used in the Newmaster *et al*. (2013) paper, is the most complex of these procedures and involves several stages: DNA extraction and purity confirmation, PCR of the DNA barcode regions, DNA sequencing and computer analysis. Whilst the initial stages could be performed in a relatively modest laboratory with inexpensive equipment, DNA sequencing would normally be carried out by an external service provider and the sequence analysis would require an experienced molecular biologist. We suspect that few companies will be able to DNA barcode their own materials in the near future, and would need to sub-contract their DNA barcode analysis.

2. PCR testing of source plant materials

DNA barcoding is appropriate for use as a gold standard for verification of species identity, but it is not yet a robust tool for routine industrial quality control procedures. To address this need, we have developed a range of simple, more reliable, PCR tests that target individual differences in DNA barcode sequences (Howard *et al*., 2009). For example, our collaboration with Dr Willmar Schwabe Pharmaceuticals (funded by the European Union Seventh Framework Programme [FP7/2007-2013] under grant agreement PIAPP-GA-2011-286328 VITANGO) aims to develop DNA tests to confirm the identity of batches of wild-harvested *Rhodiola rosea* roots. The preliminary design of such tests requires expert input, but their implementation is straightforward. DNA can be extracted from a range of samples, from fresh tissue to dried ground powders. A trained technician could conduct up to a dozen extractions in 2-3 hours. The DNA is then amplified by PCR and analysed by gel electrophoresis. This stage would take a further 5 hours to obtain a yes/no answer to the question, “Is the target species present in these samples?”. This process, from DNA extraction to gel imaging, could be carried out in a basic molecular biology facility that we estimate could be kitted out for less than £20,000.

Tests to identify suspected adulterant species can also be developed, including multiple species in complex mixtures (Howard *et al*., 2012). The simple PCR approach can also be adapted to obtain quantitative measurements of the amount of target DNA. This would require a real-time PCR (or qPCR) machine, with the cheapest machine costing around £10,000. Real-time PCR is actually quicker and

simpler to run than conventional PCR (because the gel electrophoresis step is not required), though the analysis of results is rather more sophisticated.

Other applications

Testing of processed products

Whilst we have demonstrated the possibility of extracting DNA from a range of processed product types (Kazi et al., 2013), we share some of the doubts expressed by Gafner *et al*. (2013) about the use of DNA barcoding as an authentication test for processed products. It is not always easy to obtain good yields of DNA from processed materials, and it may require further clean-up to remove contaminants that act as PCR inhibitors. Most importantly, processing may degrade the DNA to fragments shorter than the DNA barcode regions. For this reason, we think that the use of simple PCR tests that target very short DNA regions is more reliable than using DNA sequencing as the primary assay. Even then, the results should be treated with care, because the source plant DNA may be completely degraded during processing, and minor contamination by another species later in the process could produce misleading results.

Summary

We believe that DNA testing has an important role to play in the authentication of herbal medicines. Our group has developed a range of DNA tests targeting a variety of medicinal plant species. The group has the scope to increase our method development, and focus on new plants or products. Our aim is to develop bespoke problem-solving tools for the industry, and we welcome collaboration with a range of industrial partners.

References

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Alexandra Davis, Dr Caroline Howard and Professor Adrian Slater

Biomolecular Technology Group,

Faculty of Health and Life Sciences

De Montfort University,

Leicester, UK