IDENTIFICATION AND COMPARISON OF FUNGAL DRUGS

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ABSTRACT: The analysis of bulk samples of drugs of abuse, whether it be for the identification, quantification or comparison of different samples follows a simple basic pattern of a physical description, a colour test and where necessary, identification using one or more instrumental techniques. In order to develop a test for these fungi, it is necessary to identify parts of the DNA which are specific to genus. In order to determine this, the DNA was extracted from fungal drugs and PCR was performed on the ITS-1 region. In using this system to identify fungal material to genus, PCR was extracted on extracted DNA using a common primer and genus specific primer.

KEY WORDS: Fungal drugs; DNA; PCR.

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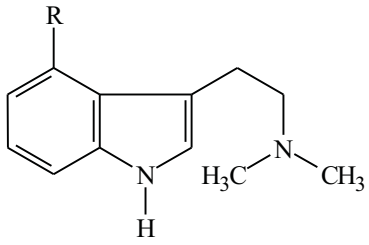
INTRODUCTION

The analysis of bulk samples of drugs of abuse, whether it be for the identification, quantification or comparison of different samples follows a simple basic pattern of a physical description, a colour test and where necessary, identification using one or more instrumental techniques. For trace samples, a physical description is generally followed directly by instrumental methods in order to obtain the maximum amount of sample from the minimum amount used. In recent times, there has been a considerable increase in drug trafficking in general, but there has been a particularly noticeable increase in the trafficking of herbal and fungal drugs. In order that control of these materials can be applied it may be necessary to identify these materials to the level of genus, or even species, depending upon the legal system in which the forensic scientist is working. Further, once the materials have been identified, comparison between samples might be made to determine whether two or more samples came from a once larger batch.

THE IDENTIFICATION OF FUNGAL DRUGS

With particular reference to fungal drugs, identification to species may not be possible on the basis of chemical content alone, since there are no species specific chemical markers. Further, the problem is exacerbated by the fact that many controlled substances within fungal material are not stable. An example of where this problem is particularly marked is that of the identification of fungi which produce the hallucinogens psilocin and psilocybin (Figure 1), both of which are not stable for long periods of time.



R = PO3 Psilocybin
R = OH Psilocin

Fig. 1. Structure of psilocybin and psilocin.

These drugs are produced by a number of genera, including *Panaeolus, Psilocybe* and *Inocybe*. The difficulty in identification to species is further exacerbated by the fact that many of these materials are encountered in powdered form, where the morphological characteristics, which are difficult to use anyway are further lost. Where morphological characteristics are not available, another method of identification to the level of species is required. One approach that can be adopted is that of the analysis of the DNA of the fungal material, where genus or even species specific markers can be identified.

In order to achieve this, it is necessary that the DNA to be examined is conserved within the genus but varies between genera. At the species level the level of variation is at that of species, with conservation within the genus. Where DNA is to be used as a marker, it should, in addition, be present in multiple copies. One target for DNA that meets these criteria is that found in the ribosomal gene complexes (rDNA) of fungi. Variation in the introns of such DNA can, in principle, be used to identify genera and species.

In eukaryotes the genes for 18S, 28S and 5.8S ribosomal RNA (rDNA) are usually arranged as tandem repeats (Figure 2).

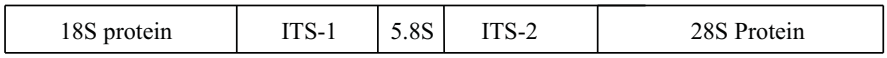


Fig. 2. Arrangement of rDNA in fungi. ITS-1 and ITS-2 represents internal transcribed sequences 1 and 2 respectively.

In fungi the number of rDNA repeats ranges from approximately 60 in the mushroom *Coprinus* to 220 in the mould *Neurospera*. These gene sequences are highly conserved, as they evolve very slowly, and are consequently used in evolutionary studies for distantly related organisms. Such conserved gene sequences are unlikely to separate or distinguish more closely related genera such as *Psilocybe* and *Panaeolus*. The rDNA gene sequences are separated by the variable Internal Transcribed Spacer (ITS) and a non-transcribed intergenic spacer (IGS). Fungi have multiple copies of this rDNA gene complex, which makes this gene locus amenable to PCR, even from highly degraded samples. The ITS region is removed after transcription and is therefore non-coding. The high degree of polymorphisms, both of sequence and length, within the ITS region has been used in the identification of fungal species.

In order to develop a test for these fungi, it is necessary to identify parts of the DNA which are specific to genus. In order to determine this, the DNA was extracted from fungal drugs and PCR was performed on the ITS-1 region using primers known to work on all fungi, the sequences of which are shown in Figure 3.

5'-GGAAGTAAAAGTCGTAACAAGG-3'

5'-GCTGCGTTCTTCATCGATGC-3'

Fig. 3. Universal primers used for the amplification of the fungal ITS-1 region.

The product is sequenced and genus specific regions are identified. Using this information, it is possible to identify sequences that can be used as templates for genus specific primers. These in turn were synthesised ready for drug identification. The sequences employed are shown in Figure 4.

Common 5'-GCTGCGTTCTTCATCGATGC-3'

Psilocybe 5'-ATATCTCCACCTGTGCACCTTT-3'

Panaeolus 5-AACGTTTCAGGTCCTATGT-3'

Fig. 4. Primers used for the identification of fungal genera by amplification of genus specific regions of the ITS-1 region.

In using this system to identify fungal material to genus, PCR was extracted on extracted DNA using a common primer (to illustrate the functionality of the PCR and subsequent analyses) and genus specific primer. The products are subsequently electrophoresed. If one band is observed, the PCR has worked but the material is not from the genus for which the specific primer is designed. If two bands are observed, the identity of the fungal material to genus has been achieved. Further development of DNA technology has now enabled fungal materials to be identified to species.

CONCLUSION

In terms of future developments of this work, it ought, in principle, be possible to develop models for the identification of a fungal species from a large number of DNA profiles obtained using such technologies. One of the difficulties posed is the use of models where amplified products are absent – effectively a zero value. New models and techniques need to be developed to deal with such data.