Forensic Science International
112 (2000) 123–133

www.elsevier.com/locate/forsciint

Identification of members of the genera *Panaeolus* and *Psilocybe* by a DNA test
A preliminary test for hallucinogenic fungi

James Chun-I Lee[a], Michael Cole[b], Adrian Linacre[b,*]

[a]*Department of Forensic Science, Central Police University, 56 Shu-Jen Road, Kwei-San, Taoyan, 33334, Taiwan*
[b]*Forensic Science Unit, Department of Pure & Applied Chemistry, Strathclyde University, Royal College, 204 George Street, Glasgow G1 1XW, UK*

Received 8 December 1999; received in revised form 13 March 2000; accepted 22 March 2000

Abstract

Abuse of hallucinogens produced by the fungal genera *Psilocybe* and *Panaeolus* are a growing problem. Five species from each of the two genera were examined in this preliminary research and a method that will unambiguously identify fungal samples as being of one of these two genera has been developed. The method uses genus specific DNA sequences within the Internal Transcribed Spacer of the ribosomal gene complex. Amplification of a common DNA product and a genus specific product results in two identifiable products, which facilitates the unambiguous identification of material from these two fungi to generic level.

Keywords: Hallucinogenic fungi; *Psilocybe*; *Panaeolus*; Psilocybin; Psilocin; ITS-1

---

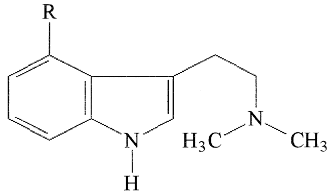
1. Introduction

Fungi of the genera *Psilocybe* and *Panaeolus* are well known to contain the hallucinogenic indole alkaloids psilocybin **1** and psilocin **2**, and as such are open to substance abuse.

---

*Corresponding author. Tel.: +44-141-548-4377 or +44-141-548-2100; fax: +44-141-548-2532.
E-mail address: a.m.t.linacre@strath.ac.uk (A. Linacre).

PII: S0379-0738(00)00181-X



1 R = Phosphate Psilocybin
2 R = OH Psilocin

In the UK, these compounds are controlled as Class A drugs under Part 1 of Schedule 2 to the Misuse of Drugs Act, 1971. Additionally, hallucinogenic mushrooms, while not in themselves controlled, are illegal in the UK if they are ‘prepared’ in a manner that puts the hallucinogens into a form suitable for consumption – for example drying. Similar legislation is documented in other legal systems [1].

Fungal samples commonly encountered in forensic investigations are often small, and may be powdered. The unambiguous identification of controlled fungal material based on morphological characteristics such as gill structure or spore shape may often not be possible. The development of a test that identifies the presence of these hallucinogenic fungi based on DNA polymorphisms may be advantageous. The DNA isolated from such seizures may be degraded and of low molecular weight. In such cases the polymerase chain reaction (PCR) [2] can be used to detect DNA polymorphisms.

In eukaryotes the genes for 18S, 28S and 5.8S ribosomal RNA (rDNA) are usually arranged as tandem repeats. In fungi the number of rDNA repeats ranges from approximately 60 in the mushroom *Coprinus* [3] to 220 in the mould *Neurospera* [4]. These gene sequences are highly conserved, as they evolve very slowly, and are consequently used in evolutionary studies for distantly related organisms [5]. 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) [5]. Fungi have multiple copies of this rDNA gene complex, which makes this gene locus amenable to PCR, even from highly degraded samples [6]. 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 [7–11] and separation of species into pathogenicity groups [12]. In some fungal genera, such as *Colletotrichum*, the ITS locus varies by only a few nucleotides [13]. Species within other genera have been shown to be more variable [14].

In this study we report on the isolation and complete sequencing of the ITS-1 locus from five members of the fungal genera *Psilocybe* and *Panaeolus*. Sequence data from this region was used to design a DNA based test that would identify unambiguously the presence of the ten members of these two genera. Commercially available fungal species were not detected. More members of each genus will be tested to ensure the specificity of the technique.

2. Material and methods

2.1. Samples

Samples of *Psilocybe* sp. and *Panaeolus* sp. were collected from different habitats in Scotland and identified using morphological characteristics. The samples form part of a collection at Strathclyde University originally collected in 1981 and stored as dehydrated samples. The samples represented five species from the genus *Psilocybe* and five from the genus *Panaeolus*. Voucher specimens are deposited at the Forensic Science Unit, Strathclyde University, with the reference numbers as assigned in Table 1.

2.2. Isolation of genomic DNA

DNA was extracted using the CTAB method [15]. Samples were ground to a powder in a mortar and pestle. The addition of a trace of sand assisted in the production of fine powder. The powder was transferred to a 1.5 ml microcentrifuge tube to which 0.5 ml extraction buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, 100 mM EDTA, 1% SDS) was added. The sample was gently shaken for 1 h at room temperature. To the solution 75 μl of 6 M NaCl was added and the solution mixed, then 65 μl of 10% CTAB/NaCl solution was added and the sample mixed then incubated at 65°C for 10 to 20 min. The solution was chloroform extracted by adding 0.5 ml chloroform/isoamylalcohol (24:1 v/v) and the aqueous layer extracted to a fresh tube. The DNA was precipitated with isopropanol, washed in 70% ethanol and resuspended in 0.5 ml TE. RNase (1 μl of 10 mg/ml) was added and the samples incubated overnight at 4°C. The samples were reprecipitated by ethanol and finally resuspended in 100 μl of TE buffer.

2.3. Amplification of ITS-1

Amplification was performed in a 50 μl volume containing 15 ng of genomic DNA, 5 μl 10×reaction buffer (100 mM Tris–HCl pH 8.8, 15 mM $MgCl_2$, 15 mM KCl, 0.1% Triton X-100), 200 μM dNTPs, 1 unit Dynazyme™ II DNA Polymerase (Finnzymes Oy, Finland,) and 0.5 μM of each primer. The primers used were the common ITS primers described by White et al. [5] being (i) 5′-GGAAGTAAAAGTCGTAACAAGG-3′ and (ii) 5′-GCTGCGTTCTTCATCGATGC-3′. Amplification was performed in a PE Biosystems (Foster City, CA, USA) 480 thermal cycler using the following cycling: 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min for 35 cycles.

Table 1
Species of *Psilocybe* and *Panaeolus* used in this study

Sample	Species	Sample	Species
PS1	*Psilocybe semilanceata*	PA1	*Panaeolus semilovatus*
PS2	*Psilocybe coprophila*	PA2	*Panaeolus papillonaceus*
PS3	*Psilocybe cyanescens*	PA3	*Panaeolus reckenii*
PS4	*Psilocybe eucalypta*	PA4	*Panaeolus speciosus*
PS5	*Psilocybe montana*	PA5	*Panaeolus subalteatus*

2.4. DNA sequencing

Approximately 50 ng of purified PCR product was sequenced using the ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, CA, USA). Primers used in the PCR were used in the sequencing reactions to sequence from both 5′ termini. Separation of sequencing products was performed on a PE Biosystems PRISM 310 Genetic Analyzer according to the manufacturer's instructions.

2.5. Data analysis

Sequence data was compared using the Pileup and Genetic distance programmes of GCG (Genetics Computer Group).

2.6. Amplification of genus specific PCR products

According to the sequencing data, the genus specific PCR was designed for quick screening of the *Psilocybe* and *Panaeolus* genera. Amplification was performed exactly as for ITS-1. The primers used were:

5′ GCTGCGTTCTTCATCGATGC 3′	Common to both genera
5′ TCCGTAGGTGAACCTGCGG 3′	Common to both genera
5′ ATATCTCCACCTGTGCACCTTT 3′	*Psilocybe* specific
5′ AACGTTTCAGGTCCTATGT 3′	*Panaeolus* specific

The primers were chosen on the basis of the sequence data and would either amplify both genera or were designed to be genus specific.

2.7. Agarose electrophoresis

PCR products were separated on 3% agarose gels (FMC, Rockland, ME, USA), containing 0.5 μg/ml ethidium bromide, in TBE buffer (0.89 M Tris–HCl, 0.889 M borate, 0.002 M EDTA pH 8.0) at 100 V for 30 min. PCR products were visualised by 312 nm UV light and photographed using Polaroid 667 film.

3. Results

3.1. Amplification of ITS-1 region

Amplification of the ITS-1 region from the five *Psilocybe* samples and five *Panaeolus* samples produced a variety of band sizes when separated on an agarose gel. A consistent size for the *Panaeolus* samples was produced. Greater variability within the *Psilocybe* genus was noted with sizes ranging approximately from 300 to 350 bp. Sequencing of the ITS-1 PCR products produced more accurate data; the sizes of the PCR products are shown in Table 2.

Table 2
The variation in size in base pairs of the ITS-1 amplification products, sequencing of the DNA fragments produced the exact sizes.

Sample	Size (bp)	Sample	Size (bp)
PS1	343/344	PA1	307
PS2	300	PA2	307
PS3	321	PA3	308
PS4	321	PA4	310
PS5	298	PA5	323/324/325

3.2. Sequence data from the ITS-1 locus

The sequence data obtained from the fungal samples are given in Fig. 1. Four of the *Panaeolus* species examined revealed unique sequences. The samples from *Panaeolus papilionaceus* (PA2) and *Panaeolus speciosus* (PA4) shared the same sequence even though they come from two different species. The five species of *Psilocybe* genus all exhibited unique sequences. To show comparison to agriculturally produced fungi, a sample of *Volvariella volvacea* was analysed in the same way. Sequence and Pileup data is shown in Fig. 1. Genetic distances generated by Kimura 2-parameter method [16] produced by the Distance software in GCG are shown in Table 4. A dendrogram (Fig. 2) generated by genetic distance data clearly separates the two fungi genera into their respective groups indicating that ITS-1 sequences can separate *Panaeolus* species from *Psilocybe* species. Within the *Panaeolus* genus the greatest genetic distance between two species (*Panaeolus semilovatus* and *Panaeolus subalteatus*) was 6.13. Within the *Psilocybe* genus the greatest genetic distance between two species (*Psilocybe semilanceata* and *Psilocybe montana*) was 22.77. These data give a clear indication of the close genetic relationship of the species within these genera, especially in *Panaeolus*.

Comparison of the sequence data to the EMBL database showed that the closest relationship of any *Panaeolus* species (PA1) to any other described species was for *Tomentella sublilacina* at 73.6%. The greatest similarity of any *Psilocybe* species (PS1) was 78.3% to *Dermocybe oliaceopicta*. The phylogenetic study of these two fungi genera is shown in Figs. 2 and 3.

The sequencing results of ITS-1 locus showed that all the species tested, except *Psilocybe semilanceata* (PS1) and *Panaeolus subalteatus* (PA5), had homologous

Table 3
The size of genus specific DNA fragments amplified from the ITS-1 DNA in nuclear ribosomal RNA gene by common primer and specific primers

Sample	Common fragment (bp)	Specific fragment (bp)	Sample	Common fragment (bp)	Specific fragment (bp)
PS1	319/320	225/226	PA1	283	133
PS2	276	183	PA2	283	133
PS3	297	204	PA3	284	133
PS4	297	204	PA4	286	133
PS5	274	181	PA5	299/300/301	136/137

```
     1                                                 50
Cons GGAAGTAAAA GTCGTAACAA GGTTTCCGTA GGTGAACCTG CGGAAGGATC

     51                                               100
PA5a ---------- -----.a--- ---------- ---------- ----------
PA5b ---------- -----.a--- ---------- ---------- ----------
PA5c ---------- -----.a--- ---------- ---------- ----------
PA5d ---------- -----.a--- ---------- ---------- ----------
PA2  ---------- -----.a--- ---------- ---------- --------c-
PA4  ---------- -----.a--- ---------- ---------- --------c-
PA1  ---------- -----.a--- ---------- ---------- ----------
PA3  ---------- -----gg--- ---------- ---------- ----------
PS1a -----t---- g-----g-c- c------a-- --gt------ -----c--.-
PS1b -----t---- g-----g-c- c------a-- --gt------ -----c--.-
PS3  -----t---- --ct--g-cg t------a-- --gt------ -----c--.-
PS4  -----t---- --ct--g-cg t------a-- --gt------ -----c--.-
PS2  -----t---- ----c-gatg t--------- --a-t--t-- ---a-t--.-
PS5  -----t---- ----c-gatg t------a-- --g-t----- ---a-t--.-
VO   ----ca---- cg-acgc--g tc-ggc-gat --ctggctc- tc--agcag-
Cons ATTATCGAAT AAACTT-GGT GGGTTGTTGC TGTCCCTCTC GGGGGAATTG

     101                                              150
PA5a -----a---- -----t---- ---------- ---------- ----------
PA5b -----a---- -----t---- ---------- ---------- ----------
PA5c -----a---- -----t---- ---------- ---------- ----------
PA5d -----a---- -----t---- ---------- ---------- ----------
PA2  ---------- -----a---- ---------- ---------- ----------
PA4  ---------- -----a---- ---------- ---------- ----------
PA1  ---------- -----t---- ---------- ---------- ----------
PA3  ---------- ---c-t---- ---------- ---------- ----------
PS1a ---t----g- gt-a-c---a -c-c------ -------ctt t-----ac--
PS1b ---t----g- gt-a-c---a -c-c------ -------ctt t-----ac--
PS3  ---t----.c gt-a-c---a -a-c------ -------ctt t-----acg-
PS4  ---t----.c gt-a-c---a -a-c------ -------ctt t-----acg-
PS2  --------.c gt-a-c---a -a-c------ -------ctt t-----ac--
PS5  --------.c gt-a-c---a -a-------- -------ctt t-----ac-c
VO   --------c- c--cgacgcc --ccattct- cac-tc-c-- -ctgt-ca-c
Cons TGCACGCCTT ACCTT-TTTG TTTTTCCACC TGTGCACACA CTGTAGGTCT

     151                                              200
PA5a -a-------- ---------- tt----a-c- tc-------- --c-------
PA5b -a-------- ---------- tt----a-c- tc-------- --c-------
PA5c -a-------- --.------- tt----a-c- tc-------- --c-------
PA5d -a-------- --.------- tt----a-c- tc-------- --c-------
PA2  ...----t-- -----a--ca ---ccc--a- .......... ..g-------
PA4  ...----t-- -----a--ca ---ccc--a- .......... ..g-------
PA1  ...------a -----a--ca ---ccc--a- .......... ..g-------
PA3  ...----t-- -----a--ca ---ccc--a- .......... ..g-------
PS1a ---tt--tta -ctttcc-a- gaaac-cggt -aagag--tt gc---cacaa
PS1b ---tt--tta -ctttcc-a- gaaac-cggt -aagag--tt gc---cacaa
PS3  t--aact--a t---a-a--- ------cctt -aagt----g g-----tc--
PS4  t-gaact--a t---a-a--- ------cctt -aagt----g gt----tc--
PS2  ----att--a g--ta..... .......... ......atct -t---cttt-
PS5  a-.-cgattt g--ca..... .......... ......atc- ag-ca-tt--
VO   ttct-ta--c cgt-aa-cc- c--c---cgg -t----.... .cggc--tac
Cons GGAGGAGGGG AAGGGGGGGG ACTTGTTA-C C-CCCTGAAA CATTTTCAGG
```

Fig. 1.

```
     201                                              250
PA5a .......... .........- --------t- ---------c ----------
PA5b .......... .........- --------t- ---------. ----------
PA5c .......... .........- --------t- ---------c ----------
PA5d .......... .........- --------t- ---------. ----------
PA2  .......... .........- --------.- ---------. ----------
PA4  .......... .........- --------.- ---------. ----------
PA1  .......... .........- -------t.- ac-------. ----------
PA3  .......... .........- -------c.- ---------. ----------
PS1a ---------- -a-------c -aggcc--cg --ttcatat- -c-c-aag--
PS1b ---------- -.-------c -aggcc--cg --ttcatat- -c-c-aag--
PS3  c......... .......... ..gc-c--cg --ttcatat- -c-c-aag--
PS4  c......... .......... ..gc-c--cg --ttcatat- -c-c-aag--
PS2  -......... .......... ..gcctatg- ---tcatat- -c-c--agt-
PS5  -......... .......... ..cc-acga- ---tcatat- -c-c-.agt-
VO   -aga--t--t gt-caccct- gag-aaa--g -gttg--g-g tgt-c--gt-
Cons GCTCTCCTTG C-AATTAACT CCTATGTA-T TTACACATAA CACTATTAAA

     251                                              300
PA5a ---------- ---------- ---t--c--- ---------- ----------
PA5b ---------- ---------- ---t--c--- ---------- ----------
PA5c ---------- ---------- ---t--c--- ---------- ----------
PA5d ---------- ---------- ---t--c--- ---------- ----------
PA2  ---------- ---------- ---.------ ---------- ----------
PA4  ---------- ---------- ---.------ ---------- ----------
PA1  ---------- ---------- ---.------ ---------- -a--------
PA3  ---------- ---------- ---.------ ---------- ----------
PS1a t--------- t-ta-t-tat t-g-ct-gt- ---------- ----------
PS1b t--------- t-ta-t-tat t-g-ct-gt- ---------- ----------
PS3  t--------- t-ta.tct-a t-g--t--t- ---------- ----------
PS4  t--------- t-ta.tct-a t-g--t--t- ---------- ----------
PS2  t--------- t-ta.tc-at --g--tcgt- ---------- -a--------
PS5  t---tt---- t-ta.tc-at --g--tcgt- ---------a t---------
VO   c-accgg-g- -ccc--gtc- -c--ca--ga -a--cc-... ..--------
Cons AGTAACAGAA CGATTCAATG GGCCTCTAAG CCTATAAACT ATATACAACT

     301                                         345
Cons TTCAGCAACG GATCTCTTGG CTCTCGCATC GATGAAGAAC GCAGC
```

Fig. 1. Sequence alignment by Pretty program of GCG software. The left column is sample name, VO is *Volvariella volvacea*, and Cons stands for consensus sequences automatically extracted by the same program. Symbol '.' is deletion, '-' is the same sequence with the consensus sequence. The five blocked sequences indicate the primer positions to amplify this ITS-1 DNA fragment and genus specific DNA fragments described in the text. Positions 1–22 and 326–345 are the fungal primers described by White et al. [5]. The second of these is also common primer 1. Between nucleotides 25 and 43 is the second common primer. The *Psilocybe* specific primer nucleotides are between 120 and 131. The *Panaeolus* specific primer is at position 190–127.

nuclear ribosomal RNA gene sequences (Fig. 1). *Psilocybe semilanceata* (PS1) sample produced two DNA fragments due to a single-base deletion at nucleotide position 212. These two DNA fragments were named PS1a and PS1b. There were four DNA fragments amplified from *Panaeolus subalteatus* (PA5) sample due to deletions at nucleotide positions 163 and 240; these four fragments were named PA5a, PA5b, PA5c and PA5d. Although there were four alleles found three sizes were produced. The three DNA fragments of 299, 300 and 301 bp were detected and confirmed in the genus specific PCR amplification and automated sequencing (Table 3).

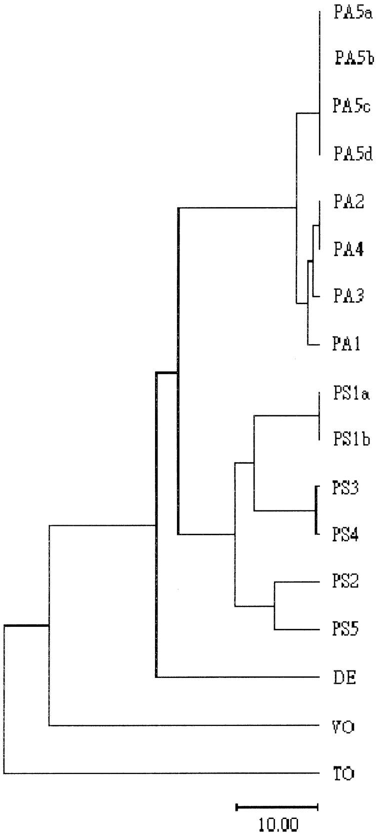


Fig. 2. Dendrogram established by the genetic distances in Table 4 produced by the Growtree software in GCG. VO is *Volvariella volvacea*, DE is *Dermocybe oliaceopicta* and TO is *Tomentella sublilacina*.

3.3. Design of genus specific primers

The DNA sequence from the ITS-1 region was examined for the presence of genus specific sequences. The alignment of the five *Psilocybe* sp. and five *Panaeolus* sp. DNA sequences showed regions of DNA sequence that were present in either of these two

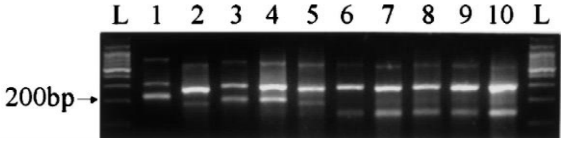


Fig. 3. PCR products of ITS-1 locus simultaneously amplified by common primers and *Psilocybe* and *Panaeolus* genus specific primers. L is 100 bp ladder and lanes 1–10 are sample PS1, PS2, PS3, PS4, PS5, PA1, PA2, PA3, PA4, PA5, respectively. The specific products of each genus were described in Table 3.

Table 4
Genetic distance matrix of 17 nucleotide sequences generated by the Kimura 2-parameter method [16] produced by the Distance software in GCG, sequence No. 1–14 are the same as the sequences in Fig. 2 from PA5a to PS5, sequence No. 15–17 are the ITS-1 DNA sequences of *Tomentella sublilacina*, *Dermocybe oliaceopicta* and *Volvariella volvacea*, two sequences are compared to each other and distances are estimated number of substitutions per 100 bases, scores of 0.00 indicated the sequences are the same, higher scores indicate less similarity in sequence, sequences within the same genus consistently produced low scores but comparison between *Psilocybe* species and agriculturally grown species produced higher scores

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0.00	0.00	0.00	0.00	5.41	5.41	6.11	5.76	39.83	39.83	33.59	34.60	32.39	35.49	73.83	47.38	66.83
2		0.00	0.00	0.00	5.41	5.41	6.11	5.76	39.48	39.48	33.25	34.25	32.02	35.12	73.33	47.60	66.40
3			0.00	0.00	5.43	5.43	6.13	5.78	39.48	39.48	33.73	34.74	32.52	35.64	73.33	46.98	67.18
4				0.00	5.43	5.43	6.13	5.78	39.12	39.12	33.38	34.39	32.15	35.27	72.82	47.21	66.74
5					0.00	0.00	2.65	1.65	34.28	34.28	31.36	31.36	29.74	31.01	71.28	41.62	62.77
6						0.00	2.65	1.65	34.28	34.28	31.36	31.36	29.74	31.01	71.28	41.62	62.77
7							0.00	2.66	36.36	36.36	32.39	32.39	28.76	33.15	70.31	42.87	61.21
8								0.00	34.64	34.64	31.73	31.73	29.65	30.84	73.84	42.08	62.43
9									0.00	0.00	16.30	16.68	18.87	22.77	83.42	38.86	71.06
10										0.00	16.30	16.68	18.87	22.77	83.94	38.49	70.64
11											0.00	0.63	16.76	19.46	81.93	33.92	71.55
12												0.00	16.77	19.45	79.79	32.87	71.54
13													0.00	11.20	71.84	25.18	59.95
14														0.00	82.84	27.47	65.56
15															0.00	105.08	91.28
16																0.00	83.93
17																	0.00

genera but not present in the other species. The sequences are highlighted in Fig. 1. As a control for the amplification process common primers were also used that would produce an amplification product from all fungi. The positions of the genus specific and common primers are illustrated in Fig. 1. These primer sets were tested on the five *Psilocybe*, five *Panaeolus* species, and a range of commonly occurring fungi. In each amplification a common product is produced but a second genus specific fragment was only produced by *Psilocybe* and *Panaeolus* samples. The *Psilocybe* genus specific product varied in size from 181 to 226 bp (Table 4). The genus specific *Panaeolus* PCR product varied in size from 133 to 137 bp. A gel image with the PCR products is shown in Fig. 3.

4. Conclusion

Ribosomal DNA ITS sequence data from five members of two fungal genera, *Psilocybe* and *Panaeolus*, which produce controlled substances, has been produced. The DNA sequence from the members of the genus *Panaeolus* showed little variation in sequence of length, whereas members of the genus *Psilocybe* exhibited greater variation.

DNA sequences within the ITS locus that were conserved within each genus, were used to produce a DNA based test that will identify the presence of hallucinogenic fungi and will further characterise the genus of fungi present. The test incorporates a common PCR set that will always produce a PCR product in the presence of fungal material, preventing a false positive being identified. The presence of a PCR product using the genus specific primers will unambiguously identify the presence of a controlled fungal species. The size of the ITS product is also indicative of the species present. Seized fungal material that can not be identified by morphological means and where the chemical hallucinogenic compounds have degraded, can be identified by this novel DNA test.

References

[1] R. Fortson (Ed.), Misuse of Drug and Drug Trafficking Offences, Sweet and Maxwell, London, 1996, p. 146.
[2] K.B. Mullis, F. Faloona, S.J. Scharf, R.K. Saiki, G.T. Horn, H.A. Ehrlich, Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction, Cold Spring Harbor Symp. Quant. Biol. 51 (1986) 263–273.
[3] J. Cassidy, D. Moore, B. Lu, P. Pukkila, Unusual organization and lack of recombination in the ribosomal RNA genes of *Coprinus cinereus*, Curr. Genet. 8 (1984) 607–613.
[4] P. Russell, S. Wagner, K. Rodland, R. Feinbaum, J. Russell, M. Bret-Haret, S. Free, R. Metzenberg, Organisation of the ribosomal ribonucleic acid genes in various wild-type strains and wild-collected strains of *Neurospera*, Mol. Gen. Genet. 196 (1984) 275–282.
[5] T.J. White, T.D. Bruns, S.B. Lee, J.W. Taylor, Amplification and Direct Sequencing of Fungal Ribosomal Genes for Phylogenetics, in: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), PCR Protocols, Academic Press, San Diego, CA, USA, 1990, pp. 315–322.
[6] T.D. Gardes, T.D. Burns, ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts, Mol. Ecol. 2 (1993) 113–118.

[7] B. Henrion, F. Le Tacon, F. Matrin, Rapid identification of genetic variation of *ectomycorrhizal* fungi by amplification of ribosomal RNA genes, New Phytologist 122 (1992) 289–298.

[8] B. Henrion, G. Chevalier, F. Martin, Typing truffles species by PCR amplification of the ribosomal DNA spacers, Mycol. Res. 98 (1994) 37–43.

[9] A. Dresler-Nurmi, S. Kaijalainen, K. Lindström, A. Hatakka, Grouping of lignin degrading corticioid fungi based on RFLP analysis of the 18S rDNA and ITS regions, Mycol. Res. 103 (1999) 990–996.

[10] M. Briad, M. Dutertre, F. Rouxel, Y. Brygoo, Ribosomal RNA sequence divergence within the Pythiaceae, Mycol. Res. 99 (1995) 1119–1127.

[11] F. Buscot, D. Wipf, C. Battista, J.-C. Munch, B. Botton, F. Martin, DNA polymorphisms in morels: PCR/RFLP analysis of the ribosomal DNA spacers and microsatellite-primed PCR, Mycol. Res. 100 (1996) 63–71.

[12] V.M. Morales, L.E. Pelcher, J.L. Taylor, Comparison of the 5.8S rDNA and internal transcribed spacer sequences of isolates of *Leptosphaeria maculans* from different pathogenicity groups, Curr. Genet. 23 (1993) 490–495.

[13] S. Sreenivasaprasad, A.E. Brown, P.R. Mills, DNA sequence variation and interrelationships among *Colletotrichum* species causing strawberry anthracnose, Physiol. Mol. Plant Pathol. 41 (1992) 265–281.

[14] C. Neuveglise, Y. Brygoo, B. Vercambre, G. Riba, Comparative analysis of molecular and biological characteristics of strains of *Beauveria brongniartii* isolated from insects, Mycol. Res. 95 (1994) 19–30.

[15] J.J. Doyle, J.I. Doyle, Isolation of plant DNA from fresh tissue, Focus 12 (1990) 13–15.

[16] M. Kimura, A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences, J. Mol. Evol. 16 (1980) 111–120.