IDENTIFICATION OF HALLUCINOGENIC AND TOXIC FUNGI BY RANDOM AMPLIFIED MICROSATELLITES (RAMS)

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The identification of hallucinogenic mushrooms is of importance in order to prove and control illegal trafficking. In addition to their availability through the illegal market, many users choose to pick their own mushrooms - a dangerous hobby that often results in poisoning due to misidentification. In such cases, fast identification of the mushroom species is fundamental for the determination of the severity of the exposure. Currently DNA sequencing of the internal transcribed spacer (ITS) region of the rDNA is the most reliable and successful DNA-based approach for the identification of fungi. However, while sequencing is a very popular method in research studies, it is not commonly used in forensic labs where the use of microsatellite-detecting methods is the preferred choice. In 1996, Hantula *et al*. described the use of Random Amplified Microsatellites (RAMS) as a universal method to study genetic variation in fungal species. In this technique, a single 5'-degenerate 'universal' PCR primer targets the intrinsic microsatellite repeats and the DNA between the distal ends of two closely positioned microsatellite regions is amplified, resulting in species-specific amplicon patterns after gel electrophoretic separation. Since the knowledge regarding most fungal genomes is limited, a method based on universal fungal primers has a practical use in the forensic field. Here we describe a modified version of Hantula's approach for the fast and reliable identification of hallucinogenic and toxic fungi to species level. DNA was extracted from mushrooms and stored using Whatman FTA cards. Two 2mm FTA discs were punched from each card, purified and placed in separate PCR reaction tubes for amplification, using the respective fluorescently labelled, degenerate "universal" primer (5'-6FAM-degenerate CCA repeat or 5'-6FAM-degenerate CGA repeat). The fragments were separated and analysed with the ABI 310 Genetic Analyzer and GeneMapper version 4.0 (Applied Biosystems). The preliminary analyses using closely related mushroom species, showed species-specific RAMS profiles were generated for each primer used. Concatenation of the profiles generated by the individual primers showed distinct patterns that could identify with confidence the individual species. DNA sequencing was performed to confirm the fungal species being tested. Further analysis of additional hallucinogenic and toxic species is under way and will be used to create a species identification database of RAMS profiles for forensic use.