B130 Botanical DNA Evidence in a Case of Illegal Drug Trafficking: The Use of High-Resolution Melting (HRM) With the Internal Transcribed Spacer (ITS) Approach in the Identification of Psychedelic Fungus (*Psilocybe sp.*)

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After attending this presentation, attendees will better understand how molecular biology techniques such as real-time Polymerase Chain Reaction (PCR) strategies, analysis of HRM curves, and DNA barcoding can help identify psychedelic fungus in illegal crops.

This presentation will impact the forensic science community by demonstrating how the results of coupling real-time PCR analysis with a DNA barcoding approach provided elements that were used in a drug trafficking court case in Chile.

Currently, as supporting evidence, DNA analysis is practically the only element that can be used as a reliable identification tool, due to the high variability of DNA nature across all species. One way to identify a distinctive DNA fragment for a species is the study of PCR products analyzed via real-time PCR. One of the most popular sequences of forensic interest at the generic and intra-generic levels in plants is the ITS. ITS refers to the spacer DNA situated between the small-subunit ribosomal RNA (rRNA) and the large-subunit rRNA genes in the chromosome or to the corresponding transcribed region in the polycistronic rRNA precursor transcript. ITS1 is located between the 18S and 5.8S rRNA genes, while ITS2 is between the 5.8S and 25S rRNA genes in plants.

Real-time PCR has many advantages over other molecular techniques since it does not require electrophoretic analysis. With real-time PCR, it is possible to distinguish PCR products using their melting Temperature (Tm) curves via differential analysis. In nature, there are more than 200 species of fungi with hallucinogenic properties. These fungi are classified as *Psilocybe*, *Gymnopilus*, and *Paneolus*. They contain active principles with hallucinogenic properties, such as ibotenic acid, psilocybin, psilocin, or baeocystin.

In Chile, fungi seizures are mainly composed of mature specimens or spores; however, it was found that clandestine laboratories processed fungus samples at the stage of mycelium. In this transient stage of growth (mycelium), traditional taxonomic identification is not feasible, making it necessary to develop a new method of study.

The case described in this presentation refers to the genetic analysis of mycelia of psychedelic fungi collected from a clandestine laboratory. The identity of fungus species was achieved using an ITS and HRM analysis approach. A genetic match was confirmed between the HRM curves obtained from the mycelia (evidence) and biological tissue extracted from the fungus' cap (the *Psilocybe sp.* mushroom, which served as a control). Therefore, mycelia recovered from the evidence and the fungus control were genetically indistinguishable. This HRM strategy enabled the molecular traceability of the psychedelic fungus and proved the usefulness of this approach for the identification of closely related species. The suspect was convicted of drug trafficking.

Forensic Botany, Psychedelic Fungus, High-Resolution Melting