## A96 Using Metabolomics to Gain a Deeper Understanding of Human Decomposition

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**Learning Overview:** After attending this presentation, attendees will understand how data intensive analytical techniques such as metabolomics can be used to identify unique features and profiles associated with different stages of human decomposition.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by demonstrating that metabolomics is an innovative tool capable of advancing the understanding of human decomposition rates by monitoring the metabolic changes in a variety of matrices (soil, decomposition fluids, and insect larvae) associated with human cadavers.

Recent advances in Liquid Chromatography/Mass Spectrometry (LC/MS) have allowed metabolomics to be a viable approach for monitoring dynamic biological processes, including those relevant to forensic science. Not only can LC/MS-based metabolomics discover biomarkers for specific metabolic processes, the technique also detects thousands of molecules in a single analysis that can inform about the global metabolic state of the system.

Human donors with no outward trauma were placed on the soil surface at the University of Tennessee Anthropology Research Facility (ARF) and allowed to naturally decompose. Soil samples from the Cadaver Decomposition Island (CDI), as well as control soils from outside the CDI, were collected at regular intervals tied to temperature and time (Accumulated Degree Hours [ADH]). When present, decomposition fluid and larval samples were collected until cadavers completed active decay, as determined by cessation of purged decomposition fluid from the trunk.

Each collected sample was flash frozen in liquid nitrogen and stored at -80°C until extraction. Soil samples from a single time point were homogenized prior to freezing. Decomposition fluids were collected using sterile syringes when visibly pooling on the soil surface. Insect larvae masses were collected at representative development stages, and care was taken to ensure that enough individuals would remain to avoid disrupting the colony. Metabolites were extracted from all matrices using modified versions of known methods,[1] and analyses were performed using an Ultra High-Performance Liquid Chromatography-High-Resolution Mass Spectrometry (UHPLC-HRMS) by adapting the method from Lu et. al,[1] onto an Exactive™ Plus Orbitrap™ Mass Spectrometer.

While metabolomics has emerged as a powerful analytical tool for increasingly larger studies, problems have arisen during the data processing and analysis stages, as most of the common processing programs and workflows require batching samples to manage the computational efforts. However, using batches to analyze metabolomics data sets can also introduce problems when comparing data among samples due to computational variations in peak detection and alignment both within and between batches.[2,3] This is especially relevant for studies conducted over multiple years, such as this project, and attempts to overcome this issue and build a database of metabolite features associated with stages of human decomposition will be discussed.

Samples collected from 14 donors yielded >3,000 samples subjected to metabolomic analysis. Samples were placed in batches at random for extraction and analysis; however, replicate samples were analyzed on the same day. The number of injections in each batch (day) averaged around 63, and in total 58 batches have been analyzed. Results from these samples yielded several thousand spectral features between all matrices. Of these features, 2%–5% have been identified based on an in-house library of ~450 metabolites.

The known metabolite list from the Quality Control (QC) samples were used to identify a set of metabolites conserved across the majority of samples, which were then used to perform the alignment across all batches. Once all samples were properly aligned, all features were subjected to standard statistical analysis, specifically Partial Least Squares Discriminant Analysis (PLSDA) to identify features (or a set of features) unique to each sample, matrix, or donor. Further machine learning algorithms using only the list of known metabolites from soil samples identified several metabolites highly correlated with either the decomposition or control soil. Specifically, the random forest algorithm predicted control and decomposition samples with 93% accuracy in a test sample, which was not used in the model fitting process, and chose pantothenate, creatine, taurine, xylitol, xanthosine, and hypoxanthine as important indicators for classifying decomposition soils.[4] Further work is in progress to incorporate the unknown features and the other matrices into these analyses as well as correlation to the decomposition time course using ADH.

**Reference(s):**

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2. Pang, Zhinqiang et al. MetaboAnalystR 3.0: Toward an Optimized Workflow for Global Metabolomics. *Metabolites* 10:186 (2020).
3. Liu, Qin et al. Addressing the batch effect issue for LC/MS metabolomics data in data processing. *Scientific Reports* 10:13856 (2020).
4. Hastie, T. et al. The elements of statistics learning: data mining, inference, and prediction. Springer Science & Business Media (2009).

**Metabolomics, Forensic Chemistry, Machine Learning**



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