H84 Bacteria as a Postmortem Interval (PMI) Clock? Successive Bacterial Colonization of Pork and Its Implications for Forensic Investigations

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After attending this presentation, attendees will understand the nature of bacterial colonization of a cadaver with time and appreciate how, in the absence of expensive high-throughput next generation sequencing, it is possible to use Terminal Restriction Fragment Length Polymorphism (T-RFLP) and proteomics techniques to identify bacterial taxa from decomposing animal and, by extension, human tissue.

This presentation will impact the forensic science community by providing data from one of the first attempts to find a cheaper and more easily accessible, culture-independent alternative to high-throughput techniques to establish a "microbial clock" for PMI estimations. This is the first study to identify Vibrionaceae as abundant on decomposing pork.

The accurate estimation of PMI is one of the most important and difficult tasks in forensic science, particularly of decomposed cadavers.[1] The decomposition process is driven by microorganisms, enzymes, and bacteria enteric and external to the body, but to date, little is known about the potential of bacterial identification to be used as a "postmortem clock," for estimating the PMI of unknown human remains.

To investigate the succession of bacterial populations in the decomposition process, three replicate pork loins were left to decompose for a total of 60 days in the University of Huddersfield's outdoor decomposition facility. Pork was chosen in order to establish a baseline of bacterial succession on which to build with further porcine and eventually human studies. Uniform porcine muscle was chosen in order to eliminate potential variations caused by factors intrinsic to the deceased individual, and external factors, such as insect colonization, microbial composition of soil, and local and seasonal variations were minimised. Bacterial communities from the surface of the pork were swabbed at regular intervals and sequenced using T-RFLP of the 16S rDNA to identify bacterial taxa. Previous research has highlighted a dynamic shift from aerobic bacterial colonization at low PMIs to anaerobic activity at higher PMIs, but these have relied on high-throughput techniques such as 454 Pyrosequencing or Illumina®.[2,3] T-RFLP is proposed as a cheaper and more accessible alternative to next generation sequencing. Matrix-Assisted Laser Disorption/Ionization-Time-Of-Flight (MALDI-TOF) peptide mass fingerprinting offers another low-cost method of bacterial identification, which was investigated here, supported by Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) for sequencing. These techniques have been used to identify bacterial taxa in soil for forensic purposes, according to research, this is the first time they have been used in combination to isolate bacterial communities from animal tissue for the estimation of PMI.[4,5]

In general, a decreasing trend in bacterial diversity was evident during the first sampling time points. Eleven distinct bacterial families were identifiable at Day 0, but this figure dropped to three within 24 hours. The most abundant bacterial taxa found throughout the entire sampling period were Proteobacteria and, in particular, Gammaproteobacteria. Betaproteobacteria were mainly present in samples taken at low PMIs, and Alphaproteobacteria were mainly abundant after 15 days, which corroborates previous research.[2] Similarly, Cyanobacteria appeared at higher PMIs, again in accordance with previous studies.[6] Firmicutes were present within the first two days and reappeared in the swabs taken at 40, 50, and 60 days. Vibrionaceae (Gammaproteobacteria) was present in large amounts at almost all time points, but abundance reached a peak at five days, then tailed off gradually. Comamonadaceae (Betaproteobacteria) and Clostridiaceae (Firmicutes) were only present in samples taken up to 24 hours, which is partially contradictory to previous findings; however, it should be noted that up to 50% of the abundant bacterial phyla could not be readily identified by means of T-RFLP.[3] Proteomics results also showed similar changes throughout the time course, and appear to give better taxonomic resolution, however, this needs further study.

This study sought to establish the proof of concept that bacterial population succession can be determined using relatively low-cost methods that are readily available. It succeeded in identifying two out of three previously defined key bacterial contributors involved in the decomposition process and represents the first study to reveal Vibrionaceae as present on rotting pork at various PMIs. This study should serve as a springboard for further research targeted at the development of alternative methods for PMI estimations.

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