Review article

Investigating the Post-Mortem Interval (PMI) with Forensically Important Necrobiomes

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Abstract

Estimating the post-mortem time interval (PMI) is a crucial component of the forensic investigation and is extremely difficult for medico-legal experts to do. After death, the succession of microbes in various parts of the human body has enormous potential for predicting PMI. The human body is home to trillions of commensal microorganisms. These microbes behave in a different way when biological processes stop, which coincides with the death of an individual and the invasion of deteriorating microbes from the environment. Due to cell autolysis, which draws a variety of invasive macro- and microorganisms, human cadavers become a rich source of nutrients. The succession of microorganisms differs significantly at various stages of degradation, which can be investigated for precise PMI estimation. Necrobiome analysis has drawn a lot of attention in PMI estimation due to the development of microbial genomics technology and decrease in the price of DNA sequencing. The review article provides a summary of the various microorganism sources, their successional pattern, and analytical methods that can be used in the field of microbial forensics.

Keywords: Necrobiome, Microbial diversity, Decomposition analysis, Post-Mortem Interval (PMI), Time Since Death (TSD), Microbial Forensics.

Background

1.1 Necrobiome

The term "necrobiome" was coined to describe a group of prokaryotic and eukaryotic species that are linked to the decomposing remains of heterotrophic biomass, such as animal carrion and human corpses[1]. In other words, the necrobiome is the group of organisms involved in necromass decomposition, including how they interact with the necromass, with one another, and with the habitat and ecosystem surrounding them. Some microbes do not require oxygen in order to survive, while others do. Some prefer a hot climate, while others do not[2].

1.2 Stages of decomposition

Decomposition happens in a predictable sequence of events, and studying these events will help us understand necrobiome and how it can help us determine the PMI. Payne *et al.* classified the decomposition of foetal pig carcasses above ground into six stages. In chronological order, the phases are fresh, bloating, active decay, advanced decay, dried, and remain[3]. These stages can vary in length

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depending on the environment the corpse is exposed to, but each one attracts specific insects or microbes. The rate of decomposition of human remains varies depending on environmental and other factors. Temperature, burning, humidity, and the availability of oxygen are all environmental factors. Body size, clothing, and the cause of death are also some of the factors.

The characteristic necrobiomes and physiological alterations of a human cadaver at various stages of degradation are shown in Figure 1.

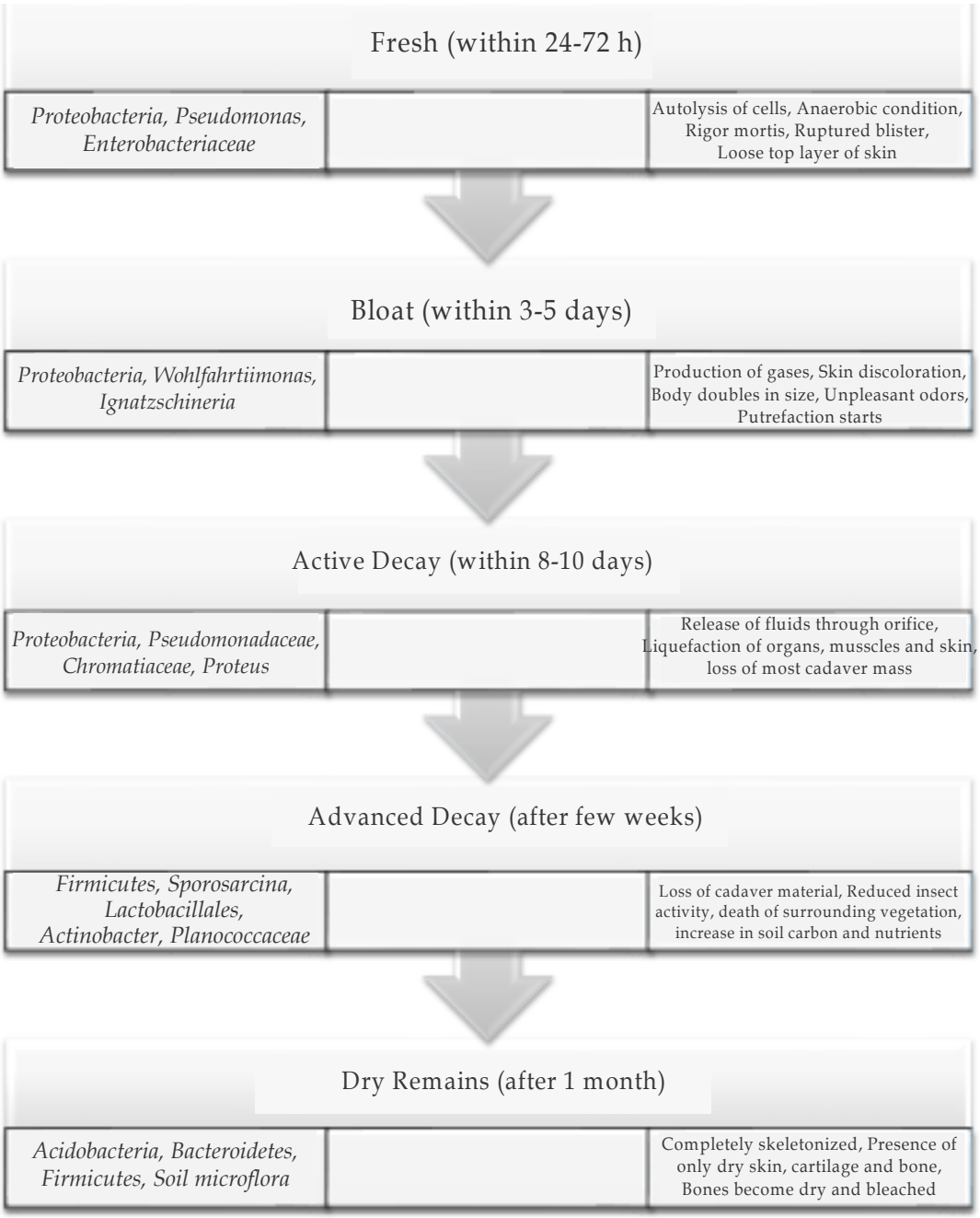

Fig 1: Overview of the signature necrobiome and physiological changes at different stages of degradation of a human cadaver

1.3 Existing techniques in Decomposition analysis

Microbial ecology technique	Study location	Study context	Key study findings	References
T-RFLP and PLFA	In-situ above ground	Soil bacteria and fungi	Successional changes in the bacterial and fungal community	4
LH-PCR	In-situ above ground	Soil bacteria	Recovered amplicons for anaerobic and nitrogen-fixing bacteria	5
454 Pyrosequencing (NGS)	In-situ above ground	Cadaver bacterial community	Shift from aerobic to anaerobic bacteria between the initial and end point of bloat stage	6
Illumina (NGS)	In-situ above ground	Soil bacterial necrobiome	Increase in TOC, PO4, and NH3; changes in functional community composition	7
454 Pyrosequencing (NGS)	In-situ above ground	Necrobiome	Bacteria members, *Ignatzschineria*, and *Wohlfahrtiimonas* spp., associated with flies recorded at bloat stage; *Actinobacter* recorded after dehydration and skeletonization	8
Illumina (NGS)	Above and below ground	Soil bacteria	Decreases in diversity, taxa richness, and evenness in surface decomposition; increased taxa richness but decreased evenness in subsurface decomposition; *Proteobacteria* dominant in both above- and belowground contexts	9
qPCR, cloning, Sanger sequencing	In situ aboveground	Gut bacterial communities	Decrease in relative abundance of *Bacteroides* and *Lactobacillus*, as potential PMI indicator	10
RT-qPCR	Proximal large intestine	Cadaver bacterial community	*Bacteroides* and *Lactobacillus* can be used as quantitative markers of PMI	10
Roche GS-FLX Titanium pyrosequencing	Lower rib	Cadaver bacterial community	99.2% of sequences were from 6 bacterial phyla: *Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria,* and *Chloroflexi*	11
Roche GS-FLX Titanium pyrosequencing	Brain, heart, liver, spleen, and blood	Cadaver bacterial community	Anaerobic *Clostridium* sp. and *Lactobacillus* sp. were the predominant bacteria	12
Roche GS-FLX Titanium pyrosequencing	Mouth, GI tract, and general body cavity, skin, and rectum	Cadaver bacterial community	A significant percentage of *Firmicutes, Bacteriodetes, and Actinobacteria* were detected. A marked shift from aerobic to anaerobic bacteria in all tissues	6
Bacterial culturing and RT-qPCR	Blood, liver, portal vein, mesenteric lymph node, and pericardial fluid	Cadaver bacterial community	21 bacteria genera were detected. The five most abundant species were: *Staphylococcus sp., Streptococcus sp., Clostridium sp., Enterococcus sp., and Escherichia sp*	13

NECROBIOME DIVERSITY

Scientists have been able to develop a "microbial clock" that can be used to determine the TSD or PMI, because changes in the microbes in and around a dead body are measurable and consistent from person to person. Scientists can estimate the time of death when a body is discovered, for example, at a crime scene, by comparing the microbes found on the body to the microbial clock. In criminal investigations, determining the time of death is crucial. It can be used to determine the actual time of murder, track down suspects at the scene, and authenticate alibis. The microbial clock technique is still in its early stages of development, and scientists are collaborating with justice practitioners to implement and integrate the new technology into the legal system.

Table 2: Depiction of different body sites for necrobiome sampling from previous literatures

BODY SITES	NECROBIOME	REFERENCES
Skin	Corynebacterium, Micrococcus, Propionibacterium, Pseudomonas, Rothia, Staphylococcus, Malassezia	14
Brain	Firmicutes, Lactobacillus, Veillonella, Prevotella, Streptococcus, Gemella	12
Oral cavity	Bacteroidetes	15,16
	Firmicutes, Proteobacteria	6,8,15
	Actinobacteria	16
	Streptococci, lactobacillus, Haemophilus, Actinomyces, Prevotella spp., Gemella spp., Veillonella spp., Granulicatella spp., Fusobacterium spp.	14
Heart, Spleen, Liver	Firmicutes, Proteobacteria, Clostridium spp., E.coli, E.albertii.	12
Small Intestine	Bacteroides, Clostridia, Streptococci, Lactobacilli, Enterococci, Gamma-Proteobacteria, E.coli	14
Large intestine	Bacteroides, Clostridia, Streptococci, Lactobacilli, Enterococci, Proteobacteria, Prevotella spp., Eubacteria, Ruminococci, Bifidobacterium, Fusobacteria.	14
Caecum	Firmicutes, Bacteroidetes	17
Ribs	Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, Chloroflexi	17

COLLECTION OF NECROBIOME FROM CORPSE

Cadaver specimens were collected by sterile swabbing of the study region, and bacterial DNA was isolated using a soil DNA extraction kit[18]. Another study examined criminal corpse cases in two ways: dissection of tissues followed by DNA extraction using phenol/chloroform and swabbing organ tissues and blood followed by heating/thawing[12]. The results of necrobiome studies showed that the swabbing method increased microbial diversity. Approaches based on 16S rRNA gene amplicon sequencing have made significant progress in opening up new research areas. These high-throughput techniques have made it possible to detect necrobiomic transition patterns that were previously undetectable using culture-based methods.

ANALYSIS OF NECROBIOMES

With an accuracy of two days or less, a TSD prediction algorithm has been created[19]. Today, forensic entomology is used in conjunction with methods for studying the necrobiome, such as DNA profiling, total soil fatty acid methyl esters, and phospholipid fatty acid (PLFA) analysis. Additionally, pig carcasses are now used as a tool to study human microbiology, which reduces the problem of variation that comes with using human cadavers as test subjects[4]. Utilizing this technology makes it easier for scientists to read the sample collection sequences. To match the name of the necrobiome, the shortened sequence is put through a database. There is a knowledge gap in various platforms throughout different parts of the world as a result of the absence of universal algorithm technology. The technology needs to be expanded in order to close that gap. However, there are some challenges, such as identifying needs, conducting research, developing prototypes, and gaining acceptance and adoption[20]. Many forensic science-related organizations would benefit from overcoming these barriers. Additionally, it would advance knowledge of the necrobiome and the growth of creating an accurate, successful multi-step experiment. The samples are put into a machine that will create and examine microbiome DNA sequences. On a computer programme in a lab, algorithms are used to read and match the sequences in the data bank. The results are returned to the most recent days within a few minutes.

TIME SINCE DEATH ESTIMATION CO-RELATION WITH NECROBIOME

There are several ways to calculate the PMI, such as by observing the contents of the mortis' stomach and its temperature. However, these methods have generally produced inaccurate results, leading to unreliable approximations. Furthermore, uncontrollable factors like environmental conditions, geographic location, and other unidentified factors have an impact on entomology[21]. Due to the diminished impact of environmental factors, biochemical methods (those based on pathophysiological changes) have been regarded as more accurate[22].

The postmortem microbiome is composed of two components: the microbial community associated with internal organs and the community associated with external body surfaces, which are known as thanato-microbiome and epinecrotic-microbiome, respectively[23]. Postmortem interval (PMI) can be inferred based on the ecological succession patterns of small organisms on the cadaver, as has long been known. For instance, PMI has been detected using the succession of species of carrion insects[24]. Complex organisms have variable developmental rates[25] and ovipositions times[26] and are frequently unavailable during specific weather or seasons[27], which places restrictions on practices of PMI estimation. As a result, microbe succession patterns have become a viable alternative to higher organisms.

CHALLENGES AND FUTURE DIRECTIONS

Although encouraging, there are a number of barriers preventing postmortem microbiome analyses from becoming standard practice in forensic investigations. For starters, most studies have been small and have used a variety of experimental techniques (e.g., DNA isolation protocol, sequencing platforms and reference databases). This lack of standardization makes it difficult to compare data from different studies. Additionally, it prevents microbiome analytics from being trusted and reliable tools in the legal system.

Investigations can also be made more difficult by temporal variations in the postmortem microbiome. Circadian oscillations in bacterial community structure have been shown in the gut and saliva, indicating that our microbiome's composition changes frequently. As a result, samples taken from the scene of a death offer a "snapshot" of the microbiome of a person at a particular point in time, but they might not accurately reflect reference samples. Similar to this, samples might include a jumble of microbes from different sources (such as a victim, a suspect, or the environment). Many crime laboratories currently lack the equipment and knowledge necessary for these kinds of analyses.

Most studies of the postmortem microbiome focus on the presence of specific microbes rather than the community's potential for function, which is true for the microbiome field as a whole. Understanding the succession of microbial species during decomposition and how they control the process are crucial. Forensic

scientists could gain a better understanding of why particular microbes are present in particular death circumstances by combining sequencing methods with those that examine mechanisms of degradation.

The development of the technologies used to study complex microbial communities will determine how well the relationship between forensic science and the postmortem microbiome develops. Microbiome analyses, however, might eventually develop into useful tools that complement more well-established investigative methods.

Conclusion

Tools with potential for forensic use include necrobiome, which is a relatively new, dynamic, and evolving technology. Estimating the TSD using necrobiome technology shows great promise for precise PMI estimation. Forensic science greatly benefits from an understanding of the dynamics and process of decomposition in order to estimate PMI accurately, but each of the methods currently in use has drawbacks. Community dynamics brought on by a variety of internal and external factors make interpretation difficult. Metagenomics applications that can resolve species- and strain-level details can offer fresh perceptions of the spatiotemporal characteristics of forensic evidence. For the estimation of PMI, the succession of microbial communities in human cadavers has produced encouraging results. However, a reliable necrobiome-based PMI prediction depends on an understanding of the repeatability of such succession under various geographical and environmental conditions. In addition, cutting-edge methods like flow cytometry can be used to determine PMI and examine the rate of DNA degradation.

A project on the human post-mortem microbiome can be started in order to collect and grow a variety of microorganisms in a post-mortem setting in order to create a post-mortem microbiome catalogue. The proposed working group ensures that a framework for microbial communities in grave soil and necrobiomes is provided. Additionally, the project's goal is to create a framework for the validation and standardization of protocols that will be used in necrobiome analysis for precise post-mortem time interval estimation.

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