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An initial investigation into the ecology of culturable aerobic postmortem bacteria

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ABSTRACT

Postmortem microorganisms are increasingly recognized for their potential to serve as physical evidence. Yet, we still understand little about the ecology of postmortem microbes, particularly those associated with the skin and larval masses. We conducted an experiment to characterize microbiological and chemical properties of decomposing swine (*Sus scrofa domesticus*) carcasses on the island of Oahu, Hawaii, USA, during June 2013. Bacteria were collected from the head, limb, and larval mass during the initial 145 h of decomposition. We also measured the pH, temperature, and oxidation–reduction potential of larval masses *in situ*. Bacteria were cultured aerobically on Standard Nutrient Agar at 22 °C and identified using protein or genetic signals. Carcass decomposition followed a typical sigmoidal pattern and associated bacterial communities differed by sampling location and time since death, although all communities were dominated by phyla Actinobacteria, Firmicutes, and Proteobacteria. Larval masses were reducing environments (~−200 mV) of neutral pH (6.5–7.5) and high temperature (35 °C–40 °C). We recommend that culturable postmortem and larval mass microbiology and chemistry be investigated in more detail, as it has potential to complement culture-independent studies and serve as a rapid estimate of PMI.

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1. Introduction

Arguably, the most rapidly developing field of forensic science is postmortem microbiology. Postmortem microorganisms are in the process of expanding from contributing primarily to establishing cause of death [1,2] to serving as physical evidence to establish the identity of an individual [3], serving as trace/associative evidence [4], and estimating postmortem interval [5,6]. To meet these ends in a reliable manner, we must understand the ecology of postmortem microbial communities; we currently have only a rudimentary grasp on the structure of postmortem microbial communities and how they interact with their environment.

The understanding of postmortem microbial communities has expanded greatly in recent years, often as a result of next-generation sequencing capabilities that were previously unavailable [e.g. 7]. It has been observed repeatedly across ecosystems, carcass taxa, and carcass sites that the postmortem microbial community is dominated by bacteria from phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria [5,6,8–11]. The taxa representing these phyla tend to be heterotrophic with the ability to colonize a resource and proliferate via the catalysis of protein and lipids [12–14]. This is an important, if unsurprising, observation since the structure of microbial communities in other habitats often includes a significant proportion of autotrophic microorganisms and/or those that metabolize polysaccharides derived from plant detritus [15]. Another recent observation is that the succession of postmortem microorganisms identified using next-generation sequencing is predictable and can be used to estimate postmortem interval [5,6,11]. All of these observations are important developments for the use of microorganisms as physical evidence in medicolegal death investigation; however, a substantial amount of research must still be conducted for these processes to have reliability sufficient for admissibility.

One conspicuous habitat for postmortem microorganisms that remains largely unexplored is the mass formed by fly larvae that have colonized a carcass, referred to here as larval mass or maggot mass. These larval masses are important components of a death investigation because the collection of fly larvae can facilitate the acquisition of many types of investigative data [see 16]. Defining the chemistry and microbiology of these masses will allow us to better understand microbial sequencing data and may enhance forensic entomology [see 17] while also serving as useful adjuncts to autopsy [1,18]. For example, we have shown that microbial communities in a larval mass change after death, and these changes appear to be associated with particular postmortem intervals [19]. Thus, these changes might serve as presumptive estimates of PMI while more confirmatory tests are conducted during subsequent weeks. These chemical and microbiological data can then

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be used to corroborate entomological estimates of PMI or even serve as tools for PMI estimation when entomological expertise or testimonial evidence is not available.

With these long-term goals in mind, we conducted an initial decomposition experiment using swine carcasses (*Sus scrofa domesticus*) on the island of Oahu, Hawaii, USA, to establish a fundamental understanding of the microbial ecology of the larval mass. The current project used techniques whereby bacteria collected from decomposing carcasses were cultured and isolated on a broad-spectrum, agar-based, growth medium incubated in aerobic conditions at room temperature (~22 °C). Thus, the short-term goal of this research project was not to provide a definitive characterization of postmortem microbial communities but rather to assess the level of insight provided by a simple, inexpensive, and rapid screening method to select for aerobic microorganisms. We conducted this experiment to test the hypothesis that the bacterial community and chemical environment of the maggot mass (pH, oxidation reduction potential, temperature) on a decomposing corpse will change as postmortem interval increases. The long-term goal of this project is to determine if these changes are predictable trends that can be used as temporal physical evidence.

2. Materials and methods

2.1. Carcasses and decomposition site

Three swine carcasses, each approximately 25 kg, were purchased from Shinsato Farms located in the Ko'olau foothills in Kaneohe, Oahu, Hawaii. Swine were killed via electrocution and transported to the decomposition site within 1 h postmortem. Electrocution can compromise the integrity of the cell membrane, a process known as electroporation, and result in entry and exit wounds if sufficient voltage is used. This is important because, like many disturbances, it can result in the ability for microbes to access nutrients that are typically not available. Although the electrocution used to kill the swine for the current study did not leave any visible damage on the carcass, it is unknown if electroporation occurred or if there was any effect on the integrity of the swine cells.

Carcasses were placed on the soil surface of a tropical savanna [20] ecosystem in the Palalo Valley, Oahu, Hawaii, in June 2013. This site is approximately 285 ft above sea level with mean annual precipitation of approximately 700 mm, 70% of which occurs in the autumn and winter (October–March). The vegetation at the site is representative of a tropical savanna ecosystem [20] on Oahu; it is rocky and dominated by pili grass (*Heteropogon contortus*) with night blooming cereus (*Hylocereus undatus*), shrub aloe (*Aloe arborescens*), and carrion plants (*Stapelia* spp.). Few scavengers are present at the site; only the small Asian mongoose (*Herpestes javanicus*) has been observed.

2.2. Experimental procedure

A datalogger (HOBO U23 Pro v2, Product #U23-001, Onset Corp., Cape Cod, MA, USA) was placed at the site to measure temperature (°C) and relative humidity (%) at intervals of 1 h (Fig. 1a, b). Following carcass placement, gross decomposition was monitored daily until 312 h postmortem. In addition, decomposition scores based on those described in Megyesi et al. [21] were assigned to each carcass. Scores were given to the head, torso, and limbs, which were then summed to produce a total body score, representing the extent of decomposition.

2.3. Identification of microorganisms

To characterize the culturable aerobic bacterial community during decomposition, the heads and limbs of the carcasses were swabbed with sterile cotton swabs (Product #25-802 2PC, Puritan Medical Products Company, Guildford, MA, USA) at 4 h, 32.5 h, 57 h, 73 h, 96 h, 120 h, and 144.5 h postmortem. Maggot masses that formed on the carcasses were also swabbed by inserting the swab into the active maggot mass. Three swabs were collected from each carcass (head, limb, larval mass) at each sampling time. Maggot masses were present from 70 h to 120 h postmortem and were swabbed twice per day from 73 h to 144.5 h postmortem with sterile cotton swabs. All swabs were placed in sterile 15 ml tubes (Product #89039-664, VWR International, West Chester, PA, USA) and transported back to the laboratory. In addition to swabbing, the chemistry of the larval mass was characterized *in situ* using a portable meter (Hach, Product #H170-G, Loveland, CO, USA) with sensors to measure temperature (ThermoWorks, Product #221-071, Lindon, Utah, USA), pH (Hach, Product #PHW77-SS, Loveland, CO, USA), and oxidation–reduction potential (Hach, Product #ORP110-GS, Loveland, CO, USA).

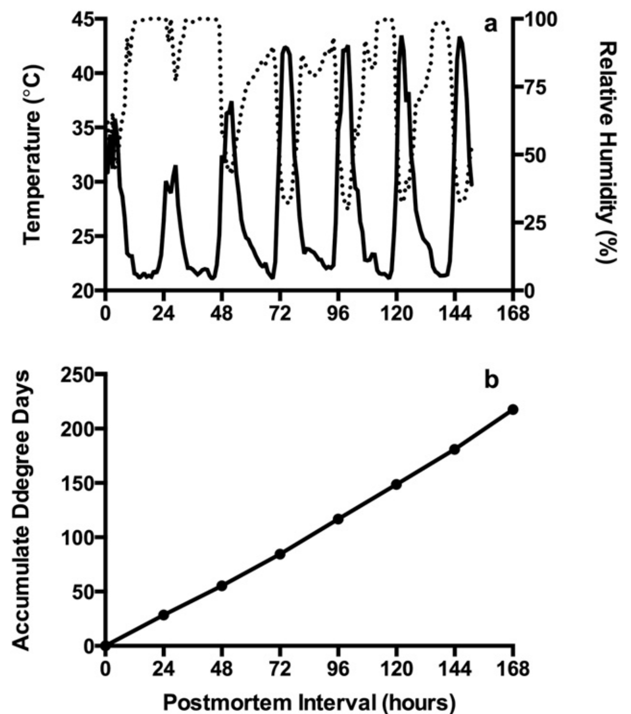

Fig. 1. Temperature (°C: solid curve) and relative humidity (%: dotted curve) during the decomposition of swine (*Sus scrofa domesticus*) carcasses placed on the soil surface of a tropical savanna ecosystem in the Palolo Valley, Oahu, Hawaii, in June 2013 (a). Temperature and relative humidity were measured at intervals of 1 h. Accumulated degree days (b) were calculated using 0 °C as the minimum developmental threshold [36].

Following delivery to the laboratory, bacterial swabs were streaked onto Standard Nutrient Agar (HiMedia Laboratories, Product #M877, Lot #161670, Mumbai, India) and incubated at 22 °C. After 48 h of incubation, each morphologically distinct bacterial colony was isolated and continued to incubate at 22 °C. These isolated colonies were identified using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) at Charles River-Accugenix (Newark, DE, USA). If MALDI-TOF was unsuccessful in determining bacterial identities, isolates were identified genetically using the 16S ribosomal RNA gene.

MALDI-TOF is adapted for use in microbiology laboratories, where it serves as a rapid and reliable method for accurate microbial identification [22]. Due to its high resolving power and sensitivity, MALDI-TOF is well suited to serve as a basis for microbial identification. The major advantage of using MALDI-TOF, a proteomic method, compared to the "gold standard" molecular methods such as 16S rRNA, lies in the cost and turnaround time from sample collection, treatment, and identification. However, MALDI-TOF depends on the assumption that sizes of peptides and smaller proteins are unique to a bacterial taxon. As a result, it is limited in the same way by reference database size as is 16S rRNA methods [23].

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