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Research Article

Estimation of Short Post-Mortem Interval Using Colony-Forming Unit (CFU) Profiling of Decomposing Fish Muscle Tissue: A Simplified Microbial Approach

Alfiya Fatima[1], Varun Jain[1], Vaishali Vardiya[1], Lakshya Bhardwaj[1], Deepak Bharti[2], Nikita Tomar[1]
[1]Faculty of Sciences, SAM Global University, Raisen-464551, Madhya Pradesh, India
[2]CMBR Biotech Pvt. Ltd., Bhopal-462022, Madhya Pradesh, India
Corresponding Author Email: deanscience@samglobaluniversity.ac.in
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Abstract

Background: Estimating the post-mortem interval (PMI) remains a significant challenge in forensic science, particularly as traditional physical indicators become unreliable with advancing decomposition. Microbial-based approaches have emerged as promising alternatives due to the predictable proliferation of bacteria during tissue breakdown.

This study aimed to investigate whether simple, culture-based enumeration of viable bacteria, expressed as colony-forming units per gram (CFU/g), could serve as a measurable indicator of short-term PMI using fish muscle as an experimental model.

Fresh fish samples were kept at ambient room temperature (approximately 25–28°C). Muscle tissue was aseptically collected at three post-mortem time points: 4, 8, and 12 hours. Tissue homogenates were serially diluted, plated on nutrient agar, and incubated at 37°C for 24–48 hours. CFU/g values were calculated from plates containing 30–300 colonies.

Even without molecular or biochemical identification, CFU-based enumeration captured a strong, time-dependent increase in bacterial load during early decomposition. This simple, low-cost method may complement traditional forensic tools for PMI estimation, particularly in resource-limited settings. Further studies using mammalian models and controlled environmental conditions are warranted.

Keywords

Post-Mortem Interval; Forensic Microbiology; Decomposition; Colony-Forming Units; Fish Tissue; Bacterial Enumeration

1. Introduction

Determining the time elapsed since death, known as the post-mortem interval (PMI), is a cornerstone of forensic investigations. Accurate PMI estimates can help reconstruct events, corroborate or challenge witness statements, and provide critical evidence in legal proceedings. Traditionally, forensic experts rely on physical changes such as algor mortis (body cooling), rigor mortis (muscle stiffening), and livor mortis (post-mortem hypostasis), along with patterns of decomposition and insect activity [1,2]. However, these indicators are highly sensitive to environmental variables, including temperature, humidity, clothing, air circulation, and scavenger access [3]. As decomposition advances, these

classical markers lose precision, necessitating alternative or complementary approaches.

Over the past two decades, microbial communities associated with decomposing remains have gained attention as potential forensic tools. Following death, the cessation of blood circulation and immune system failure allows internal bacteria, particularly gut microbiota, to proliferate and migrate. Simultaneously, environmental bacteria colonize exposed tissues [4,5]. Research has shown that both the composition and total load of bacterial communities change in structured, time-dependent patterns, leading to the concept of a "microbial clock" [6–9].

While many studies have employed high-throughput DNA sequencing (e.g., 16S rRNA gene amplicon sequencing), simple culture-based methods remain valuable. They are inexpensive, widely accessible, and require minimal infrastructure [10,11]. Among these, colony-forming unit (CFU) enumeration provides a direct measure of viable bacterial density in a given tissue sample. During decomposition, tissue autolysis releases nutrients, promoting rapid bacterial multiplication. Thus, CFU/g values are expected to increase with PMI [12,13].

Fish muscle is a practical model for early-stage decomposition studies. It is soft, protein-rich, and highly perishable, with well-characterized spoilage microbiology [14–16]. The objective of this study was to determine whether CFU counts alone, without species identification, could reveal a clear, monotonic increase in bacterial load over short post-mortem intervals (4, 8, and 12 hours) at room temperature, using fish muscle as a proxy for decomposing animal tissue.

2. Materials and Methods

2.1. Sample Collection and Preparation

Fresh, whole fish (species not specified, locally sourced) were obtained from a certified market in Bhopal, Madhya Pradesh, India, and transported to the laboratory in clean, insulated containers within 30 minutes. Only specimens with intact skin, clear eyes, and no off-odor were selected. Upon arrival, each fish was rinsed briefly with sterile distilled water to remove surface debris. Using sterile scalpels and scissors, approximately 10 g of epaxial muscle was dissected from beneath the dorsal skin to minimize surface contamination.

The tissue was divided into three portions, each representing one post-mortem sampling time: 4, 8, and 12 hours. Each portion was placed in a separate sterile container and maintained at ambient room temperature (25–28°C) without refrigeration to allow natural decomposition.

2.2. Experimental Design and Sampling

At each predetermined time point (4, 8, and 12 hours post-dissection), approximately 1 g of muscle tissue was aseptically excised from the respective container. Each sample was weighed using a sterile digital balance and transferred into a sterile mortar containing 9 mL of 0.85% (w/v) sterile normal saline. The tissue was thoroughly homogenized to obtain a uniform 10^{-1} suspension.

2.3. Serial Dilution and Plating

Serial ten-fold dilutions (10^{-2} to 10^{-7}) were prepared by transferring 1 mL of the previous dilution into 9 mL of sterile normal saline, using fresh sterile pipette tips at each step. From each dilution, 100 µL was spread onto nutrient agar plates (prepared as per standard formulation: peptone, beef extract, sodium chloride,

agar; pH 7.0; autoclaved at 121°C, 15 psi for 20 minutes). All manipulations were performed inside a laminar airflow cabinet using aseptic techniques.

Plates were incubated inverted at 37°C for 24–48 hours. After incubation, plates containing 30–300 colonies were selected for counting. CFU per gram of tissue was calculated using the formula:

CFU/g = (Number of colonies × Dilution factor) / Volume plated (mL)

where the dilution factor corresponds to the reciprocal of the dilution plated (e.g., 10^4 for 10^{-4} dilution) and the volume plated was 0.1 mL. Each sample was plated in duplicate, and the mean CFU/g was calculated per time point.

2.4. Data Analysis

No statistical modeling or species identification was performed, as the study focused solely on CFU-based quantification. Results are presented as mean CFU/g values and compared descriptively across time points.

3. Results

Bacterial growth was observed on nutrient agar plates from all three post-mortem intervals, with clear differences in colony density and number (Table 1). At 4 hours, plates from the 10^{-4} dilution showed well-separated colonies, with a mean count of 52 colonies, corresponding to 5.2 × 10 CFU/g. At 8 hours, the 10^{-5} dilution yielded a mean of 118 colonies (1.18×10^8 CFU/g). At 12 hours, the 10^{-6} dilution showed a mean of 236 colonies (2.36 × 10 CFU/g).

Table 1. Colony-Forming Unit (CFU) Counts in Fish Muscle Tissue at Different Post-Mortem Intervals

Post-Mortem Interval (hours)	Dilution Factor	Mean Colony Count (n=2)	Calculated CFU/g
4	10^{-4}	52	5.2 × 10
8	10^{-5}	118	1.18 × 10
12	10^{-6}	236	2.36 × 10

The data show a stepwise increase of approximately two orders of magnitude between 4 and 8 hours, and another one to two orders of magnitude between 8 and 12 hours. This pattern was consistent across replicate plates, indicating active bacterial multiplication accompanying tissue decomposition.

4. Discussion

This study demonstrates that simple CFU-based enumeration can capture a strong, time-dependent rise in viable bacterial load during early decomposition of fish muscle at room temperature. The observed increase, from 10 to 10 CFU/g over 12 hours, aligns with expectations from decomposition microbiology. As tissues soften and release nutrients, resident and environmental bacteria multiply rapidly [4,5,12].

Our findings are consistent with previous reports on microbial succession during decomposition. Studies on soil beneath carcasses, animal models, and spoiled meat have all documented increasing bacterial abundance with time [6,7,13,17]. In fish spoilage research, similar trends in total viable counts have been reported under ambient storage conditions [14–16].

The present work extends these observations by focusing specifically on short PMIs (≤12 hours) and using only CFU enumeration, without ancillary molecular or biochemical analyses.

From a forensic perspective, the key insight is not the exact CFU values in fish tissue, but the systematic, and measurable change in bacterial load over time. In controlled environments, CFU/g could potentially be calibrated to PMI for specific tissue types and temperatures. Even in complex real-world scenarios, bacterial load could serve as an additional line of evidence alongside traditional indicators, particularly when physical signs become ambiguous [8,9].

4.1. Limitations

Several limitations must be acknowledged. First, fish muscle is not a perfect analogue for human tissues; differences in lipid content, connective tissue, and initial microbial load may affect decomposition kinetics. Second, environmental conditions (temperature, humidity, airflow) were not rigorously controlled, reflecting realistic but variable conditions. Third, the study used a narrow time window (up to 12 hours) and only one tissue type. Fourth, no mathematical modeling or statistical validation (e.g., regression analysis) was performed to quantify the CFU–PMI relationship. Finally, bacterial species were not identified, so potential differences in growth rates or metabolic activities among taxa were not considered.

Despite these limitations, the study provides proof-of-concept that CFU-based enumeration alone can reveal a strong, time-linked increase in bacterial load during decomposition. This low-cost, accessible method could be particularly useful in resource-limited forensic settings.

4.2. Future Directions

Further research should extend the time window (e.g., 0–72 hours), include more frequent sampling points, and systematically vary temperature and humidity. Applying the same CFU approach to mammalian tissues (e.g., porcine or rodent models) would increase forensic relevance. Where ethically and legally permissible, validation using human cadaveric material would be the gold standard. Additionally, combining CFU data with simple species identification (e.g., Gram staining or selective media) or mathematical modeling could enhance predictive accuracy.

5. Conclusions

This study shows that total viable bacterial load, measured as CFU/g, increases markedly and consistently in decomposing fish muscle over short post-mortem intervals (4–12 hours) at room temperature. The method is simple, inexpensive, and requires only basic microbiological equipment. While not a standalone solution, CFU-based enumeration could complement traditional forensic indicators for PMI estimation, particularly in early decomposition stages or in settings where molecular tools are unavailable. With further validation in animal models and controlled environments, this approach may become a practical addition to the forensic microbiology toolkit.

Declarations

Ethics Approval and Consent to Participate:
Not applicable. This study used commercially available fish tissue and involved no live animals or human subjects.

Consent for Publication:
All authors have reviewed and approved

the final manuscript and consent to its publication.

Data Availability:
The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request.

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Author Contributions:

- Alfiya Fatima: Conceptualization, investigation, writing – original draft.
- Varun Jain: Supervision, methodology, writing – review & editing.
- Vaishali Vardiya: Investigation, data curation.
- Lakshya Bhardwaj: Formal analysis, validation.
- Deepak Bharti: Resources, laboratory support.
- Nikita Tomar: Writing – review & editing, project administration.

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