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**REVEALING MICROBIAL COMPONENTS IN BIOFILM ON AQUATIC**

**INSECT CADAVERS: AN EXPERIMENTAL TAPHONOMIC STUDY**

**Supplementary Information Material and Methods**

A Gram stain was performed for the identification of bacteria. The Gram stain is used to observe bacterial cell morphology and provide an initial differentiation of bacteria. Bacteria that appear purple are considered Gram-positive, while those appearing pink, red, or berry-like are classified as Gram-negative. Additionally, various culture media were employed for the identification of microorganism groups.

The Gram staining procedure involved making smears for each treatment. Smears were spread on microscope slides, heat-fixed, stained with Crystal Violet for 1 minute, washed with water, covered with iodine solution (Lugol) for 1 minute, rewashed, decolorized with acetone, and finally stained with Fuchsin for 1 minute. The dried samples were then observed under an optical microscope.

Different culture media were utilized for identification:

1. MacConkey Broth is a selective and differential medium that isolates Gram-negative enteric bacilli based on lactose fermentation.
2. EC Medium isolates total coliforms, fecal coliforms, and Escherichia coli. Lactose is the carbohydrate that promotes the growth of fecal bacteria.
3. Manitol Salt Agar is used for the isolation and differentiation of staphylococci. Mannitol is the fermentable carbohydrate, and sodium chloride is the selective agent.
4. Eosin Methylene Blue (EMB) is selective for Gram-negative bacilli, differentiating lactose/sucrose fermenters from non-fermenters.

Each selective medium was prepared and sterilized by autoclaving for 15 minutes at 1 atm pressure. They were then plated under a laminar flow hood for subsequent microbial inoculation. The PCA (Plate Count Agar) medium was placed in five labeled Petri dishes corresponding to the five treatments. After solidification, microbial samples were streaked onto the agar surface using a sterile loop (five streaks, one for each treatment). This procedure was performed under sterile conditions using a Bunsen burner. The plates were then incubated at 36°C for 48 hours. Colonies originating on PCA were subjected to Gram staining, and subsequent subcultures were performed on the previously prepared selective media. These were incubated at 36°C for 48 hours, all conducted under sterile conditions.