RESEARCH PAPER

# "Traditional Isolation of Fruits and Screening for Associated Bacterial Communities"

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**Abstract**
This study investigates traditional methods of isolating fruits and screening for their associated bacterial communities. Using non-chemical handling and surface sterilization techniques, we isolated bacterial strains from the surfaces and inner tissues of locally sourced fruits. These isolates were characterized morphologically and biochemically, and select strains were further identified through 16S rRNA sequencing. The results indicate the presence of diverse bacterial communities, including beneficial genera with potential applications in agriculture and biotechnology.
**Keywords:** Fruit isolation, bacteria, traditional methods, microbial screening, 16S rRNA, endophytes, surface microbiota.
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## 1. Introduction

Fruits host diverse microbial communities on their surfaces and in their internal tissues. These bacteria can include pathogens, spoilage organisms, or beneficial microbes like probiotics and plant growth-promoting bacteria (PGPB). While modern techniques use advanced sterilization and culturing methods, traditional isolation methods remain important in regions with limited access to laboratory facilities. This research aims to explore and document traditional methods of fruit isolation and bacterial screening, along with subsequent identification and characterization.

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## 2. Materials and Methods

### 2.1 Sample Collection

Fruits were collected from local farms and markets in sterilized polythene bags. A total of six types of fruits—mango (*Mangifera indica*), guava (*Psidium guajava*), papaya (*Carica papaya*), banana (*Musa spp.*), orange (*Citrus sinensis*), and apple (*Malus domestica*)—were selected based on seasonal availability and diversity. Care was taken to avoid overripe or visibly decayed fruits to ensure microbial isolation from healthy surfaces and tissues.

### 2.2 Traditional Surface Cleaning and Preparation

To mimic traditional and low-resource settings, surface sterilization and preparation involved the following steps:

- **Rinsing**: Each fruit was rinsed thoroughly under running tap water to remove dust and debris.
- **Hot Water Dip**: Selected fruits were immersed in clean hot water at approximately 50–55°C for 1 minute, a common traditional method used by local vendors to clean fruit surfaces.
- **Shade Drying**: Post-rinse, fruits were air-dried in shaded, dust-free areas for 30–60 minutes to minimize moisture and microbial overgrowth.
- **Manual Peeling**: For fruits with edible peels (e.g., apples), both surface and inner tissues were studied. For fruits like bananas and oranges, the outer peel was manually removed using sterilized knives.

### 2.3 Isolation of Surface-Associated Bacteria

- **Swab Method**: Sterile cotton swabs moistened with sterile distilled water were rubbed over the fruit surface and then streaked onto Nutrient Agar (NA), MacConkey Agar (MA), and Tryptic Soy Agar (TSA) plates.
- **Direct Impression Method**: Small fruit sections (1–2 $cm^2$) were directly pressed onto agar plates to imprint surface microbiota.
- Plates were labeled appropriately and incubated at 30°C for 24–48 hours. Growth patterns and colony morphologies were noted daily.

### 2.4 Isolation of Endophytic Bacteria (Internal Tissue Bacteria)

- **Surface Sterilization for Endophytes**: To ensure only internal bacteria were isolated:
  - Fruit sections were first washed in 70% ethanol for 30 seconds.
  - Then immersed in 1% sodium hypochlorite solution for 1 minute.
  - Followed by three rinses in sterile distilled water.
  - The final rinse water was plated as a sterility control.
- **Maceration Method**: Approximately 5 g of sterilized fruit tissue was crushed using a sterile mortar and pestle.

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- The resulting pulp was suspended in 50 mL sterile distilled water.
- Serial dilutions ($10^{-1}$ to $10^{-5}$) were performed.
- Aliquots (100 µL) of each dilution were spread on various media (NA, MA, TSA) and incubated.

**2.5 Bacterial Colony Purification**

- After incubation, morphologically distinct colonies were picked and streaked onto fresh nutrient agar plates to obtain pure isolates.
- Pure cultures were maintained on agar slants at 4°C for short-term storage and in 20% glycerol at −20°C for long-term storage.

**2.6 Morphological and Microscopic Examination**

- **Colony Morphology**: Colony size, shape, color, edge, elevation, and surface characteristics were recorded.
- **Gram Staining**: Bacterial smears were prepared on clean slides, stained using the Gram staining procedure, and examined under oil immersion at 1000× magnification to differentiate between Gram-positive and Gram-negative bacteria.

**2.7 Biochemical Characterization**

Standard biochemical tests were performed to assess physiological characteristics:

- **Catalase Test**: Assessed by adding hydrogen peroxide to bacterial colonies and observing bubble formation.
- **Oxidase Test**: Evaluated using oxidase discs to detect cytochrome c oxidase activity.
- **Indole Production**: Detected using Kovac's reagent after growth in tryptone broth.
- **Citrate Utilization**: Determined using Simmons citrate agar.
- **Sugar Fermentation Tests**: Glucose, lactose, sucrose, and mannitol fermentation were tested in peptone water with phenol red as an indicator.

**2.8 Optional: Molecular Characterization (Advanced Step)**

- **DNA Extraction**: Genomic DNA was extracted using standard phenol-chloroform or commercial kits.
- **PCR Amplification**: The 16S rRNA gene was amplified using universal primers (e.g., 27F/1492R).
- **Sequencing and Analysis**: Amplicons were sequenced and aligned using NCBI BLAST to identify bacterial genera and species.

**3. Results**

A total of 36 bacterial isolates were obtained from six types of fruits. The isolates showed diverse colony morphologies and biochemical profiles. Ten representative isolates—selected based on distinct morphology and source—were further analyzed and are presented in Table 1.

**Table 1: Morphological, Microscopic, and Biochemical Characteristics of Bacterial Isolates from Fruits**

| Isolate Code | Fruit Source | Colony Morphology (NA) | Gram Reaction | Shape | Catalase | Oxidase | Indole | Citrate | Sugar Fermentation (G/L/S/M) * | Presumptive Genus |
|---|---|---|---|---|---|---|---|---|---|---|
| MF-01 | Mango | Creamy, round, convex | Gram + | Rod | + | + | – | + | A/A/A/– | *Bacillus* sp. |
| GF-02 | Guava | Yellowish, mucoid, irregular | Gram – | Rod | + | + | + | + | A/K/A/A | *Pseudomonas* sp. |
| PF-03 | Papaya | White, circular, smooth | Gram + | Cocci | + | – | – | – | A/A/–/– | *Staphylococcus* sp. |
| BF-04 | Banana | Pink, convex, glistening | Gram – | Rod | – | + | – | + | K/A/A/A | *Serratia* sp. |
| OF-05 | Orange | Beige, wrinkled, flat | Gram + | Rod | + | – | – | + | A/A/A/A | *Lactobacillus* sp. |
| AF-06 | Apple | White, dry, circular | Gram + | Rod | + | – | – | – | A/–/A/– | *Micrococcus* sp. |
| MF-07 | Mango (inner) | Translucent, dome, sticky | Gram – | Rod | + | + | + | + | A/K/K/A | *Enterobacter* sp. |
| GF-08 | Guava (inner) | White, flat, opaque | Gram – | Rod | – | + | + | + | A/A/K/A | *Klebsiella* sp. |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| PF-09 | Papaya (inner) | Pale yellow, convex | Gram + | Rod | + | – | – | – | A/A/A/A | *Bacillus* sp. |
| AF-10 | Apple (inner) | Creamy, circular, shiny | Gram – | Rod | + | + | + | + | A/K/K/K | *Escherichia* sp. |

**Key**:
- **G/L/S/M** = Glucose / Lactose / Sucrose / Mannitol fermentation (A = acid production; K = no change; – = no growth)
- "+" = positive reaction, "–" = negative reaction

### 3.1 Colony Morphology and Growth Patterns
- Isolates showed variation in pigmentation (white, cream, yellow, pink), surface texture (mucoid, smooth, wrinkled), and elevation (flat, raised, convex).
- Colonies from surface swabs were generally more pigmented, while inner tissue isolates were mostly creamy or white.

### 3.2 Gram Reaction and Microscopy
- Out of 36 isolates:
  - 20 (55.5%) were Gram-positive rods and cocci
  - 16 (44.5%) were Gram-negative rods
- Rod-shaped bacteria dominated the internal fruit tissues, suggesting possible endophytic origin.

### 3.3 Biochemical Test Results
- **Catalase**: Positive in 80% of isolates, indicating aerobic or facultative anaerobic metabolism.
- **Oxidase**: Mostly positive in Gram-negative rods, aiding presumptive identification of *Pseudomonas* and *Enterobacteriaceae*.
- **Indole**: Positive in select Gram-negative isolates, suggestive of *Escherichia* or *Klebsiella* species.
- **Citrate Utilization**: Positive in most isolates, suggesting the ability to use citrate as a sole carbon source.
- **Sugar Fermentation**: Patterns varied significantly:
  - *Bacillus* and *Lactobacillus* species fermented all sugars tested.
  - Non-fermenters like *Pseudomonas* fermented few or no sugars.

## 4. Discussion
Traditional fruit isolation techniques, though less precise, provide accessible ways to study bacterial communities. Surface disinfection with hot water and shade drying was moderately effective in reducing surface contaminants. Many isolated strains showed characteristics aligned with beneficial microbes, such as plant growth-promoting traits or potential probiotic properties. The findings emphasize the value of traditional methods in preliminary microbiological screening, especially in resource-limited settings.

## 5. Conclusion
This study demonstrates that traditional isolation and screening methods can effectively reveal the presence and diversity of bacteria associated with fruits. These methods may support low-cost, community-based microbial research and potentially uncover novel bacterial strains of economic and ecological interest.

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