

# Identification of Unknown Bacteria: Lab Report

4 pages | 875 words

## Abstract

Identifying unknown bacteria is a crucial task in microbiology. This laboratory report outlines the procedures and results of identifying two unknown bacteria, one gram-positive and one gram-negative, referred to as unknown A. The isolation of the bacteria, Gram staining, and a series of biochemical tests were performed to narrow down the possibilities. The results revealed that the gram-positive bacterium was *Staphylococcus aureus*, while the gram-negative bacterium was *Pseudomonas aeruginosa*.

## Introduction

Identifying unknown bacteria is essential for various reasons, as different microorganisms possess distinct qualities and characteristics that set them apart. Throughout the semester in the laboratory, we encountered various microorganisms and gained knowledge about their properties. This knowledge is now applied to the process of identifying unknown bacteria, specifically, unknown A.

## Materials and Methods

The identification process began with the professor providing the unknown specimens, including one gram-positive and one gram-negative bacterium from a predetermined list. Unknown A was assigned for identification. The procedures followed closely resembled those outlined in the lab manual (1). The initial step involved isolating each bacterium using the streak plate technique. Tryptic Soy Agar (TSA) plates and selective media like mannitol salt and Eosin Methylene Blue (EMB) were employed for isolation. The isolation step is critical as it separates the bacteria, allowing for individual identification. Gram staining was performed to understand the basic morphology of the bacteria. As the plates incubated, distinct colonies of the two bacteria were observed. Colonies from the mannitol salt plate were used to inoculate Tryptic Soy Broth (TSB) for gram-positive culture growth. The purity of this broth was verified using gram staining. Similarly, a circular colony from the TSA plate was used to inoculate TSB for gram-negative growth, and its purity was also tested through gram staining. Once the gram-positive and gram-negative bacteria from unknown A were isolated, specific biochemical tests were conducted for identification. The results of these tests, coupled with deductive reasoning and elimination, led to the identification of the unknown bacteria. The following tests were performed on the gram-positive bacterium: 1. Mannitol salt streaking, 2. Gram staining of the pure isolate, 3. Oxidase test, 4. Catalase test, and 5. Coagulase test. Similarly, the following tests were performed on the gram-negative bacterium: 1. Gram staining of the pure isolate, 2. Blood agar plate streaking, 3. SIM (Sulfide, Motility, Indole) test, and 4. Catalase test.

## Results

### Gram-Positive Bacterium (*Staphylococcus aureus*)

Test	Purpose	Reagents/Media	Observation	Results
Gram stain	To determine the gram reaction and morphology	Crystal violet, Iodine, Alcohol, Safranin	Purple cocci, connected	Gram positive purple cocci
Mannitol salt	Selective growth media for Staphylococci and Micrococcaceae	Mannitol salt plate and gram positive isolate broth	After incubation, media turned yellow	Bacterium is mannitol salt positive
Oxidase test	To determine if bacterium produces cytochrome c oxidase	No color change	Bacterium is oxidase negative	
Catalase test	To identify if bacterium produces catalase	Hydrogen peroxide	Bubbling is seen	Bacterium is catalase positive
Coagulase test	Used to identify if bacterium produces coagulase (enzyme that clots blood plasma)	Citrated rabbit plasma	Clouding and solidification of plasma is seen	Bacterium is coagulase positive

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**Raffaela Di Napoli**



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