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isolation and identification of some bacteria from spoiled fruits

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Abstract:
Fruits provide appropriate conditions for the growth of many microorganisms. Microorganisms ruin the fruits, changing their texture and rendering them unpalatable. Fruits are heavily spoiled by bacteria, but there is also a higher danger of damage during handling and transit. Farmers incurred significant losses as a result of spoiling. In the current investigation, bacterial isolates were isolated and identified from around five damaged fruits (banana, papaya, pineapple, apple, and grape) collected from a local market in Erbil, Kurdistan area, Iraq. Biochemical testing of the isolated bacteria was also performed.
A study was conducted to look at the presence of different bacterial species in five types of ruined fruit samples. Four types of bacteria were successfully isolated using the serial dilution-agar plating method. Gram strain results revealed that two types of bacteria recovered from banana samples were Gram positive, with long rod shapes and cocci in bunches, whereas the other two samples were Gram negative bacteria. Microscopy revealed that two bacterial colonies are composed of short rod-shaped bacteria. Biochemical investigation revealed that the two most common isolated bacteria in ruined apples were *Escherichia* sp. and *Klebsiella* sp. The other two bacteria isolates were identified as *Bacillus* sp. and *Staphylococcus* sp., respectively. Improved technology based on preservation techniques are advised for improving the quality of fruits.

Keywords: - Contaminate, spoilage, bacteria, Fruits and Identification.

Introduction:

Owing to growing knowledge of healthy eating practices, the consumption of fruit products has increased in Malaysia by over 30% over the previous few decades. Additionally, the annual loss of fruit due to spoiling is estimated to be around 20% for the local fruit production. 19.6% of Malaysia's edible food losses for that year came from the 18.9 billion pounds of fresh fruit that were lost each year to spoiling, according to Department of Statistic data (Akinmsusire, 2011). Fruits is vital for maintaining a healthy lifestyle since they are high in vitamins, minerals, and fiber. They are also a great source of antioxidants, which makes them vital for defending against a range of illnesses (Dive & Gogoi, 2021, Hasan & Zwkahar, 2020). Everyday consumption of fruits is essential for sustaining a diet that is well-balanced. The limited shelf life of fruits, along with their exposure to microbial contamination through contact with soil, dust, and water, represent significant threats to their preservation. Therefore, keeping fruits fresh longer is crucial for their consumption.

A fruit's environment is perfect for many different kinds of microorganisms, particularly bacteria, to live and grow. High concentrations of different kinds of carbohydrates, minerals, vitamins, and amino acids can be

found in the internal fruit tissues (Aneja, 2009). Any alteration in the state of food that makes it unfit for human eating is referred to as spoilage (Barth et.al., 2013). The deterioration of pectin's brought on by bacterial rotting initially softens the tissues, and finally the entire fruit may turn into a slimy sludge. Following the metabolism of starch and carbohydrates, lactic acid, ethanol, and disagreeable flavors and odors emerge (Cappuccino & Sherman, 2005). On sound, unharmed plant tissue, certain spoilage microorganisms can colonize and cause lesions (Chaudhuary et.al., 2016).

Fruit microbial infections can happen during the growing season, during harvesting, handling, transporting, storing, and marketing conditions following harvest, or even after the consumer has made a purchase. Fruits that have spoiled will taste less good or possibly be harmful to customers. Numerous studies in the past have documented the presence of various bacteria, such as Pseudomonas, Erwinia, Xanthomonas, Enterobacter, Flavobacterium, Chromobacter, Lactobacillus, Bacillus, and Clostridium, in spoilt fruits. Fruits are essential food sources for humans. They provide the body with the appropriate amounts of vitamins, lipids, minerals, and oil to support human growth and development. But fruits face significant obstacles in their quest for survival. This study examines the presence of several bacteria that cause three locally important and commercially significant fruit varieties to deteriorate and rot after harvest (Dive & Gogoi, 2021, Hasan & Zwkahar, 2020).

Methodology:

1. Sample collection:

Five different kinds of raw, unprocessed spoiled fruits banana, papaya, pineapple, apple and grape were gathered in plastic zip bags from the Erbil Government local market in the Kurdistan Region and brought to the Ministry of Health's central laboratory's biology lab for additional examination.

2. Isolation of bacteria:

The process of serial dilution was used to separate microorganisms from ruined grapes. The rotten fruits were crushed and mixed with distilled water in a pre-sterile mortar and pestle to create a suspension that was diluted in steps of [101 to 106]. For each dilution, a [100 µL] diluted fruit suspension was dispersed over nutritional agar medium NAM plates that were labeled appropriately. To promote bacterial growth, the inoculated petri plates were incubated for [24 hours at 37 °C]. To ascertain the morphology variance of bacterial growth based on distinct bacterial colonies, morphological analysis was observed. In order to preserve and utilize the bacterial isolates going forward, they were sub-cultured, maintained, and kept on NAM slants at [40 °C].

3. Macroscopic and microscopic identification:

Bergey's Manual of Systematic Bacteriology (Chukwuka et.al., 2010), was used to identify the bacterial isolates based on their morphological and biochemical features. To examine the cellular structure of separated bacteria, gram staining was used. The biochemical traits of the bacterial species were further identified by measuring their production of indole, methyl red, voges-proskauer and citrate (IMViC), (Raja et.al., 2012).

Result and Discussion:

Fruits are a natural supply of minerals, vitamins, carbohydrates, and other necessary nutrients. Fruits contain a lot of water, making them vulnerable to microorganisms, which may play a role in spoiling. In this study, bacteria species were effectively recovered from five decaying fruits (banana, apple, grape, pineapple, and papaya) from a local market in the Kurdistan region/Erbil Government/Iraq.

According to conventional bacteriology, the majority of bacterial isolates can be distinguished using a simple Gram staining procedure (Tafinta et.al., 2013). Four Gram-positive bacteria were isolated from pineapple and papaya spoiled fruit samples, whereas two Gram-negative bacteria were discovered in banana spoilt fruit (Fig.1). According to (Goldman & Green, 2006), the Gram stain reaction is based on differences in the chemical composition of bacterial cell walls. Gram-positive cells have a thick peptidoglycan layer, whereas gram-negative cells have a much thinner peptidoglycan layer that is bordered by lipid-containing outer layers. As a result, gram positive bacteria appeared purple because iodine and crystal violet accumulate in the thicker

cell wall and are not eluted by alcohol, in contrast to gram negative bacteria where the crystal violet is freely available eluted from the bacteria that produced the pink colour (Dive & Gogoi, 2021, Hasan & Zwkahar, 2020).

Table 1: Depicting the presence of various bacterial isolates from damaged fruits.

Bacterial isolates	Colony morphology	Banana	Apple	Grapes	Pineapple	Papaya	Frequency
Bacillus sp.1	White dry colony	+	-	+	-	-	4(67.7)
Bacillus sp.2	White smooth colony	-	+	+	-	-	5(85.5
Staphylococcus sp.1	Yellowish golden colony	-	-	-	-	+	1(18.8)
Staphylococcus aureus	Shiny off-white colony	-	+	-	-	+	2(36.6)
Escherichia coli	White colony	+	+	+	-	+	5(81.1)
Klebsiella sp.	Light pinkish colony	-	-	-	-	-	1(17.7)
Total	6						

Not: + represents presence of bacteria and – represents absence of bacteria

Morphological identification of bacterial isolates was investigated further based on their form, texture, and color. The primary goal of morphological identification is to classify bacterial isolates based on similar morphological characteristics. If the physical traits of the bacterial isolates matched those previously described or reported, they were classified as belonging to the same group or genus. The results demonstrated that colony development, shape, and color differed between bacterial isolates. Table 1 summarizes the morphological properties of the isolated bacteria.

Bacteria were identified as present in decaying pineapple and papaya. Based on morphological traits, all bacteria displayed a similar short rod shape in a scattered pattern. The microscopic cell morphology study of the bacteria identified as presented in papaya revealed lengthy rod and bunch forms (Dive & Gogoi, 2021, Hasan & Zwkahar, 2020).

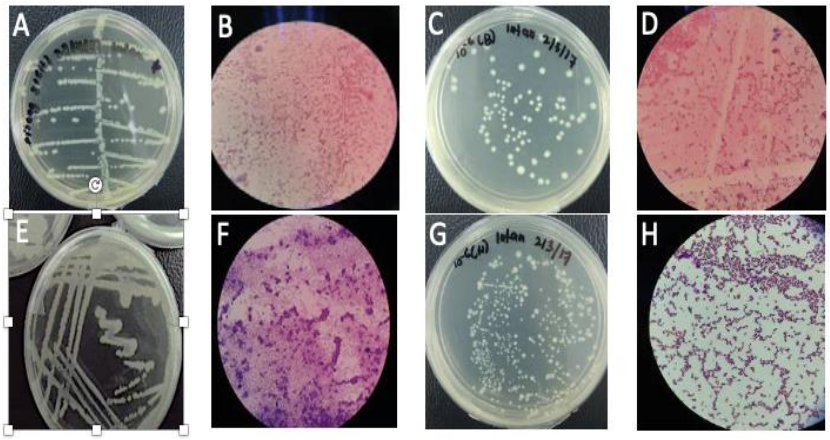

Fig. 1: - Escherichia coli colony appearance (A), Bacillus sp. colony appearance (C) (magnification 100x), negative Gram staining result (B), and Klebseilla sp. colony appearance (D). (E) Bacillus sp. colony appearance; (F) positive Gram staining result (40x magnification); (G) Staphylococcus sp. colony appearance with golden color. (H) Staphylococcus sp. stained with Gram (40x magnification).

The biochemical test results were also analyzed Table 2. *Bacillus* sp.2 and Escherichia coli were the main bacterial isolates. *Pseudomonas* sp. and B*acillus* sp. were the main bacterial isolates found in rotten apples from the local market (Raja et al., 2012). According to Chaudhary and Dhaka (2016), *Bacillus* sp. was prevalent in damaged fruits purchased from a local market. The presence of *Staphylococcus aureus*, a dangerous bacterium, in the majority of damaged fruits is a major public health concern. Similarly, the discovery of bacteria such as *Klebsiella* sp. in rotten fruits highlighted the need of human safety (Eni et al., 2010).

Table 2: Biochemical testing are used to identify bacteria in the spoiled fruits.

Bacterial Identification	Gram	Biochemical tests

	staining	Methyl red test	Catalase test	Voges Proskauer	Citrate	Indole (I)
Bacillus sp.1	Gram +ve	+	+	+	+	-
Bacillus sp.2	Gram +ve	+	-	-	+	-
Staphylococcus sp.1	Gram +ve	+	-	+	-	-
Staphylococcus aureus	Gram -ve	+	-	-	+	-
Escherichia coli	Gram +ve	+	+	-	-	-
Klebsiella sp.	Gram -ve	+	+	-	+	-

Not: + positive & - negative.

IMViC tests are commonly used to identify and differentiate Enterobacteriaceae members of the family Enterobacteriaceae. According to Table 2, all of the isolated samples tested negative for indole. According to (Tafinta, et.ai., 2013), an indole synthesis test was performed to separate organisms that generate tryptophanase, an enzyme capable of breaking the amino acid tryptophan to make indole. Methyl-Red test was developed to assess an organism's ability to make strong acid from glucose and to maintain a low pH after lengthen incubation period (Tafinta, et.al., 2012). Only bacteria on pineapple and banana yielding of red color in the medium indicated a favorable outcome with pH below 4.4 with generation of acid. Only bacterium pineapple produced a negative Voges-Proskauer test result, indicating their inability to create acetylmethylcarbinol (acetoin) through glucose metabolism. Citrate testing revealed a positive result in bacteria from pineapple and banana. This test was designed to detect an organism's capacity to use citrate as their only source of carbon and energy. Based on biochemical characteristics, isolated bacteria from three varieties of damaged apples were identified as *Escherichia coli* (pineapple), *Klebsiella* sp. *Bacillus* sp. and *Staphylococcus* sp.

Previous investigations found comparable results when *Bacillus* sp. and *Escherichia coli* were recovered from rotten papaya using serial dilution (Aneja, 2009). In contrast to (Oviaogia, et.ai., 2015 and Kumar et.al., 2012), both investigations showed *Bacillus* sp. as the major bacterial strain discovered in local and store samples of rotten fruits. *Klebsiella* sp. and *Staphylococcus* sp. were found in 30 rotten fruit samples, according to a study (Eni, et.al., 2010). The findings of this study highlight the need for safe and hygienic handling of fruits, as well as an efficient processing procedure, to prevent the growth or survival of spoilage and pathogenic microorganisms. The necessity to extend the shelf life of fruits prompted the development of fruit preservation methods. Furthermore, vegetables sold by street vendors must to ensure the quality and safety of fruits for consumers, they must be taught on food safety and hygiene procedures (Dive & Gogoi, 2021, Hasan & Zwkahar, 2020).

Conclusions:

Six bacterial species were successfully isolated from the five types of damaged fruit samples and identified as *Escherichia coli*, *Klebsiella* sp., *Bacillus* sp., and *Staphylococcus* sp. More research is needed to investigate the effect of these bacterial species on fruit spoilage. Improved preservation technology is recommended to improve the quality of fruits.

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