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Cite as: AIP Conference Proceedings **2020**, 020073 (2018); https://doi.org/10.1063/1.5062699
Published Online: 05 October 2018

N. A. Hasan and I. M. Zulkahar

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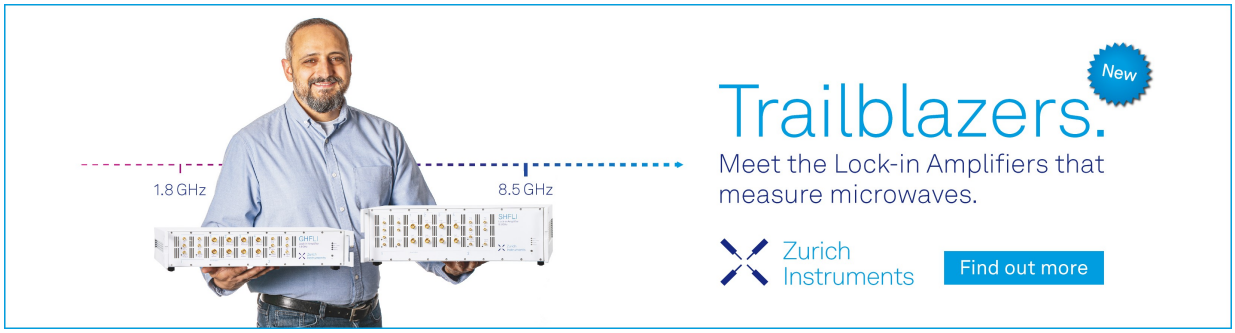

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Isolation and Identification of Bacteria from Spoiled Fruits

N. A. Hasan[1, a)] and I. M. Zulkahar[1]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA, Cawangan Negeri Sembilan, 72000 Kuala Pilah Negeri Sembilan*

[a)]Corresponding author: aishahnh@ns.uitm.edu.my

Abstract. Bacteria is the major and important factor for fruit spoilage. They will survive in suitable temperature with the presence of food and water, which caused changes in the appearance, colour and smell of the fruits. A study was carried out to examine the presence of various bacterial species in three types of spoiled fruit samples comprising of pineapple (Ananas comosus), banana (Musa paradisiaca L.) and papaya (Carica papaya). Four type of bacteria namely A1, A2, B1 and B2 were successfully isolated by serial dilution-agar plating method. Gram strain results demonstrated that B1 and B2 bacteria isolated from banana samples classified as Gram positive with long rod shape and cocci in bunches while bacterial samples A1 and A2 were classified as Gram negative bacteria. Microscopy observation showed that A1 and A2 bacterial colonies are of the short rod shaped bacteria. Biochemical analysis indicated that two isolated bacteria had the highest occurrence in spoiled fruits identified as Escherichia sp (A1) and Klebsiella sp. (A2). On the other hand, B1 and B2 isolates were identified as Bacillus sp and Staphylococcus sp. respectively. Improved technology based on preservation methods are recommended to enhance the quality of fruits.

INTRODUCTION

Consumption of fruits products has significantly increased in Malaysia by more than 30% during the past decades due to increase awareness in healthy eating habits. It is also estimated that about 20% of local fruit produced is lost each year due to spoilage. According to a Department of Statistic data [1], 18.9 billion pounds of fresh fruits were lost annually due to spoilage, which was contributed to 19.6% of edible food losses of Malaysia for that particular year.

Fruits provides the ideal environment for the survival and growth of many types of microorganisms especially bacteria. The internal fruits tissues consists high concentration of various types of sugars, minerals, vitamins and amino acids [2]. Spoilage refers to any change in the condition of food in which the food becomes undesirable or unacceptable for human consumption [3]. Bacterial spoilage first causes softening of tissues as pectins are degraded and the whole fruit may eventually degenerate into a slimy mass. Starch and sugars are metabolized next and unpleasant odours and flavours develop along with lactic acid and ethanol [4]. Some spoilage microbes are capable of colonizing and creating lesions on healthy, undamaged plant tissue [5].

Microbial fruits infection may occur during the growth season, harvesting, handling, transport and post-harvest storage and marketing conditions, or after purchasing by the consumer. Spoilage will cause fruits becomes less palatable or even toxic to consumers. Previously, several studies have been reported the occurrence of bacteria in spoiled fruits including *Pseudomonas, Erwinia, Xanthomonas, Enterobacter, Flavobacterium, Chromobacter, Lactobacillus, Bacillus,* and *Clostridium*.

Fruits are vital sources of nutrients to human beings. They give supplement the body the with necessary vitamins, fats, minerals, and oil in the right proportion for human growth and development. Fruits however, have serious challenges to their existence. This paper study the presence of various bacteria responsible for the post-harvest decay and deterioration of three economically important three types of local fruits.

Advances in Civil Engineering and Science Technology
AIP Conf. Proc. 2020, 020073-1–020073-5; https://doi.org/10.1063/1.5062699
Published by AIP Publishing. 978-0-7354-1738-0/$30.00

MATERIALS AND METHODS

Sample collection

Three types of unwashed and unprocessed spoiled fruits comprising of viz. banana, papaya and pineapple were collected in plastic zip bag from the local market of Kuala Pilah and were brought in to the Biology laboratory in UiTM Kuala Pilah for further analysis.

Isolation of bacteria

Serial dilution method was carried out to isolate bacteria from spoiled fruits. The spoiled fruits were crushed into pre-sterile mortar and pestle with distilled water to form suspension, which was serially diluted from 101 to 106 dilutions. A 100 µL of each of diluted fruit suspension was spreader over specifically labeled nutrient agar medium (NAM) plates from each dilution. The inoculated petri plates were incubated at 37ºC for 24 hours for to allow bacterial growth. Morphological analysis was observed conducted to determine based on different bacterial colonies and morphology variation of bacteria growth. Consequently, bacterial isolates were sub-cultured, maintained and stored on NAM slants at 40ºC for further use.

Macroscopic and microscopic identification

The bacterial isolates were identified based on morphological and biochemical characteristics according to the Bergey's Manual of Systematic Bacteriology [6]. Gram staining was conducted to study the cellular morphology of isolated bacteria. The bacterial species were further identified on the basis of biochemical characteristics via the determination of Indole production, Methyl-Red, Voges-Proskauer and Citrate (IMViC) [7].

Results and Discussion

Fruits are natural sources of minarels, vitamins besides carbohydrate and other essential substances. High amount of water making fruits highly prone to attack by microorganism which may play significant role in their spoilage. In this study, four type of bacteria species were successfully isolated from three rotten fruits (pineapple, banana and papaya) in Kuala Pilah local market, Malaysia. According to classical bacteriology, most species of bacterial isolate can be differentiate based on simple Gram staining technique [8]. Four Gram positive bacteria were isolate from pineapple and papaya spoiled fruit sample while two Gram negative bacteria were found in banana spoiled fruit (Fig. 1). According to [9], Gram stain reaction is based on the difference in the chemical essence of bacterial cell walls. Gram-positive cells have a thick peptidoglycan layer, whereas the peptidoglycan layer in gram-negative cells is much thinner and surrounded by outer lipid containing layers. As a result, gram positive bacteria appeared purple because iodine and crystal violet precipitate in the thickened cell wall and are not eluted by alcohol in contrast with gram negative bacteria where the crystal violet is freely eluted from the bacteria which expressed the pink colour.

Morphological identification based on the shape, texture and colour of bacterial isolates were further analyzed. The main purpose of morphological identification is to group the bacterial isolates according to similar morphological characteristics. The bacterial isolates were considered as belonging to the same group or genus if their morphological characteristics matched the morphological descriptions previously described or reported. The result showed that there is differences growth of colony, morphology and clour between bacterial isolates.Morphological characteristics of the isolated bacteria are given listed in Table 1. Bacteria labeled as A1 and A2 were presented in rotten pineapple and papaya. Based on morphological characteristics, all bacteria demonstrated a similar short rod shape in scattered arrangement. The microscopic cell morphology analysis of the bacteria labeled as B1 showed as categorized as long rod shape bacteria while bacteria labeled as B2 demonstrated a cell morphology classified as cocci in bunches shapes.

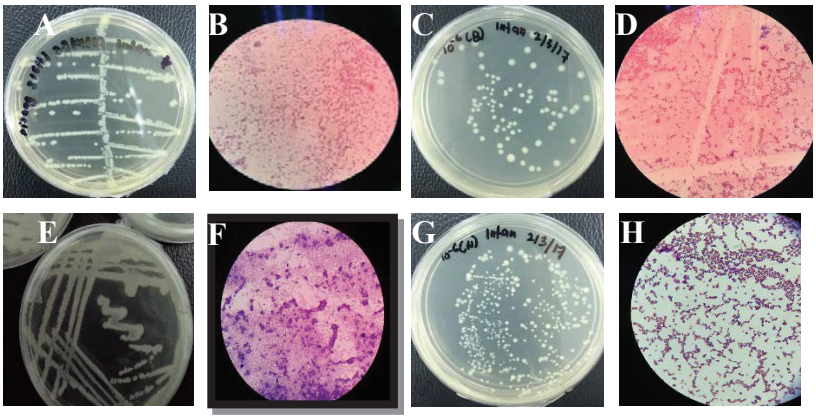

FIGURE 1.(A) Colony appearance of *Escherichia coli*, (B) Negative result of Gram staining (magnification 40x), (C) colony appearance of *Klebseilla sp.* (magnification 100x), (D) Gram staining of *Klebseilla sp.* (E) colony appearance of *Bacillus sp.,* (F) positive result of Gram staining (magnification 40x), (G) colony appearance with golden colour of *Staphylococcus* sp. (H) Gram staining of *Staphylococcus sp.* (magnification 40x).

TABLE 1. Morphological characteristics of bacteria isolated from 3 types of spoiled fruits

No.	Fruits	Isolate	Colonial morphological	Gram classification	Shape and arrangement
1	Pineapple	A1	White, opaque, large, smooth, flat, moist	-	Short rod in scattered arrangement
2		A2	White, slimy, translucent, and raise growth	-	Short rod in scattered arrangement
3	Banana	B1	White, attached spreading with crenate margin, dry	+	Long rods with round edges in scattered arrangement
4		B2	Golden yellow,circular, large, opaque, convex smooth and shiny.	+	Cocci in bunches
5	Papaya	A1	White, opaque, large, smooth, flat, moist	-	Short rod in scattered arrangement
6		A2	White, slimy, translucent, and raise growth	-	Short rod in scattered arrangement

+ positive
- negative

IMViC test are generally employed in the identification or differentiation of the Enterobacteriaceae members of family Enterobacteriaceae. Based on Table 2, all the isolated showed negative result for indole test. According to [8], indole production test was perfomed to differentiate organisms that yield tryptophanase enzyme that is able of splitting the amino acid tryptophan to produce indole. Methyl-Red test was performed to verify an organism's ability to produce strong acid from glucose and to maintain a low pH after lengthen incubation period [8]. Only bacteria A1 and B2 yielding of red colour in the medium indicated a positive result with pH below 4.4 with production of acid. For Voges-Proskauer test, only bacteria A1 showed a negative result indicated the incapability of this bacteria to produce acetylmethylcarbinol (acetoin) from glucose metabolism. Positive result was observed in bacteria A2 and B1 on citrate test. This test was conducted to detect the ability of an organisms to utilize citrate as the sole source of carbon and energy. Based on biochemical characteristic, isolated bacteria from three types of spoiled fruits were identified as Escherichia coli (A1), Klebsiella sp. (A2), Bacillus sp. (B1) and Staphylococcus sp. (B2). Previous study studies reported similar result where [10] isolated Bacillus sp. and Escherichia coli from spoiled papaya by serial dilution. Contradictory to [11] and [12], were both studies reported that Bacillus sp. dominantly bacterial

isolated bacteria found in local and supermarkets samples of spoiled fruits. Presence of Klebsiella sp. and Stapylococcus sp. have been reported in by [13] study which were isolated from 30 spoiled fruit samples.

Findings in this study suggested support the requirement of safety and hygienic handling of fruits as well as and good efficient processing method to reduce the growth or survival of spoilage and pathogenic microorganisms. The development of fruits preservation process has been driven by the need to extend shelf life of fruits. Besides, street vended fruits must be educated about food safety and hygiene practices to ensure the quality and safety of the fruits to consumers.

TABLE 2. IMViC test on isolated bacterial isolates from spoiled fruit samples

Biochemical test	**A1**	**A2**	**B1**	**B2**	**A1**	**A2**
Indole (I)	-	-	-	-	-	-
Methyl red (M)	+	-	-	+	+	-
Voges Proskauer (VP)	-	+	+	+	-	+
Citrate (C)	-	+	+	-	-	+
Bacteria identified	*Escherichia coli*	*Klebsiella sp.*	*Bacillus sp.*	*Staphylococcus sp.*	*Escherichia coli*	*Klebsiella sp.*

+ positive
- negative

CONCLUSION

Six bacterial were successfully isolated in three types of spoiled fruits samples and identified as *Escherichia coli* (A1), *Klebsiella sp.*(A2) *Bacillus sp.* (B1) and *Staphylococcus sp.*(B2). Further research work is required to explore more about the effect of these bacterial species associated with fruit spoilage. Improved technology based on preservation methods are recommended to enhance the quality of fruits.

ACKNOWLEDGEMENT

Authors feel grateful to Universiti Teknologi MARA, Malaysia for allocate facilities during research activities.

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