

ANTIMICROBIAL ACTIVITY OF LOCALLY ISOLATE BACTERIA
FROM FRUIT SKINS (MANGO AND PAPAYA)

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ABSTRACT

Nowadays, the minimal processed products have become more popular. Prevention of the growth of bacteria in cut fruits is one of the critical points in the processing steps. Characterization of bacteria from fruits and the effects of antimicrobial agents in inhibiting the growth of these bacteria are the important aspects to be studied in order to minimize the contamination of bacteria in fruits. Therefore, this study aimed to isolate the bacteria from mango and papaya fruit skins followed by the characterization of the bacteria using biochemical tests. In addition, this study also aimed to study the effect of antibacterial agent and temperature in inhibiting the growth of bacteria from fruit skins. A total of 8 different types of bacteria from mango and papaya fruit skins were successfully isolated. Then, 9 types of biochemical tests with the gram staining have been performed in the strains. As a result, M1 and M2 assign as *Bacillus*, M3 and M4 assign as *Streptococcus* or *Enterococcus*, P1 assign as *Aeromonas*, P2 as *Micrococcus* or *Staphylococcus*, P3 as *Neisseria* or *Veillonella* and P4 as *Lactobacillus*. Effect of two parameters, which were antibacterial agent (XY-12; a chlorine based agent) and temperature, in retarding the growth of bacteria in mango and papaya were investigated. The fruit skins which were treated with XY-12 at 4°C followed by incubation at 4°C were the best condition in inhibiting the growth of bacteria with almost 100 % percentage of inhibition of bacterial growth for mango and papaya, respectively. Meanwhile, fruit skins samples which were treated with XY-12 at 30°C and incubated 30°C showed the lowest percentage of inhibition.

ABSTRAK

Produk-produk yang memerlukan pemrosesan yang minimum telah menjadi semakin populer. Pencegahan pembiakan bakteri dalam buah-buahan dipotong adalah salah satu isu yang kritikal dalam langkah-langkah pemrosesan. Pencirian bakteri daripada buah-buahan dan kesan agen antimikrobial dalam menghalang pertumbuhan bakteri adalah salah satu aspek penting untuk dikaji untuk mengurangkan pertumbuhan bakteri. Oleh itu, kajian ini bertujuan untuk mengasingkan bakteri daripada kulit mangga dan betik diikuti oleh pencirian bakteri menggunakan ujian biokimia. Di samping itu, kajian ini juga bertujuan untuk mengkaji kesan agen antibakterial dan suhu dalam menghalang pertumbuhan bakteri daripada kulit mangga dan betik. Sebanyak 8 jenis bakteri daripada mangga dan kulit buah-buahan betik telah berjaya diasingkan. Kemudian, 9 jenis ujian biokimia dengan pewarnaan gram telah dilakukan bagi mengenalpasti jenis-jenis bakteri yang ada di kulit mangga dan betik. Hasilnya, M1 dan M2 dilabelkan sebagai *Bacillus*, M3 dan M4 dilabelkan sebagai *Streptococcus* atau *Enterococcus*, P1 dilabelkan sebagai *Aeromonas*, P2 dilabelkan sebagai *Micrococcus* atau *Staphylococcus*, P3 dilabelkan sebagai *Neisseria* atau *Veillonella* dan P4 sebagai *Lactobacillus*. Kesan dua parameter, iaitu ejen antibakterial (XY-12; ejen berasaskan klorin) dan suhu dalam membantutkan pertumbuhan bakteri dalam mangga dan betik telah dikenalpasti. Kulit buah-buahan yang telah dirawat dengan XY-12 pada suhu 4 ° C dan disimpan pada suhu 4 ° C adalah keadaan terbaik dalam menghalang pertumbuhan bacteria. Sementara itu, kulit buah yang telah dirawat dengan XY-12 pada 30 ° C dan disimpan pada suhu 30 ° C menunjukkan peratusan yang paling rendah perencatan.

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LIST OF ABBREVIATIONS

PCR	Polymerase Chain Reaction
EDTA	Ethylenediamine tetraacetic acid
EtBr	Ethidium Bromide
H	Hour
min	Minute
kb	Kilo base
kDA	Kilo Dalton
MW	Molecular Weight
OD	Optical density
et al.	and friends
sp	Species
°C	Degree Centigrade Celcius
1000X	1000 Times Magnification
nm	Nanometer
bp	Basepair (nucleotide)
DNA	Deoxyribonucleic Acid
g/mg/μl	gram/milligram/microgram
l/ml/μl	liter/milliliter/microliter
M/mM	Molar/Millimolar
Mm/μm	Millimeter/micrometer

CHAPTER 1

INTRODUCTION

1.1 Background

According to the U.S Food and Drug Administration (USDA) and Food and Drug Administration FDA, fresh cut-fruits refer to the products which are freshly-cut, washed, packaged and maintained the quality with the cooler system (Beaulieu *et. al*,2010). In addition, International Fresh-cut Produce Association defines that fresh cut-fruit product is the fruits, which are totally turned to usable product by trimmed or peeled and pre-packaged to serve and convenience the people by offering them not only ready to eat but still remaining the high nutrition, freshness of the fruits and the consumer able to get in low price (Corbo *et.al*, 2010).

Today's society is more concern about their healthiness and the type of food they take. Therefore, balance diet has become an important aspect. Rapid lifestyle drives people to choose healthy readily made food in order to maintain a balance diet. This factor has stimulated the growth of cut-fruits industries (Corbo *et.al*, 2010).

Fruits contain water, fiber, fat, sugar, and variety of vitamins such as vitamin A, B1, B2, B6, C, E that easily promote the growth of microorganisms (Tang *et.al*, 2012). The presence of significant amount of microorganisms affects the shelf life, freshness of

the fruits and could potentially cause foodborne disease to consumers (Beaulieu *et. al*, 2010).

One of the challenging tasks in the production of cut-fruits is the control of microorganisms in the fruit (Chand *et.al*, 1996). The contamination of the bacteria may occur in any of the points including during the plantation (bacteria from soil and contaminated water), harvesting and transportation to the factory (Corbo *et.al*, 2010). In the factory, the processing steps including cutting, slicing and dicing may also transfer bacteria from the fruit's surface to the fruit's flesh.

Therefore, antimicrobial steps need to be developed in order to inhibit the growth of bacteria eventually preventing the event of foodborne disease (Endo *et. al*, 2009). Recently in September 2012, the U.S. Food and Drug Administration (FDA) highlighted the presence of the pathogenic *Salmonella* sp. in mangoes from Agricola Daniella, a mango supplier in Mexico and warned against eating mangoes from this supplier. The FDA further described that the presence of *Salmonella* sp. might be both outside and inside of the fruit. People who are infected with *Salmonella* bacteria would develop diarrhea, fever, vomiting, and abdominal cramps 12 to 72 hours after infection. In most of the cases, the illness lasts between 4 to 7 days (<http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm319464.html>).

In addition, USDA came out with the news saying that some papayas imported from Mexico containing *Salmonella* sp. which caused 97 cases of food poisoning that 10 of the cases required hospital treatment. More than 10 samples of papayas taken from Mexico have been confirmed to be contaminated by *Salmonella* sp. (Rothschild, 2011).

This project involved the isolation, characterization of the bacteria from fruit skins (mangoes and papaya). The commercial available antimicrobial agent (food grade) supplied by a cut fruit factory was used to test their effectiveness in retarding the growth of microorganisms from the fruit skins. The findings have been useful in designing and improving the fruits preservation strategies in cut fruit industries.

1.2 Problem Statement

Nowadays, the minimal processed products including cut-fruits have become more popular. Prevention the growth of microorganisms in the fruits is one of the critical points in the processing steps. Isolation, characterization of bacteria from fruits and the effects of commercial available antimicrobial agent in inhibiting the growth of these bacteria are the important aspects in order to minimize the contamination of microorganisms in fruits.

1.3 Objective of Study

1. To isolate bacteria from mango and papaya skins
2. To characterize the bacteria using biochemical tests
3. To study the effect of antimicrobial agent and temperature in inhibiting the growth of bacteria from fruit skins

1.4 Scope of Study

The bacteria were first isolated from the skins of mango and papaya. These bacteria were then characterized using biochemical tests (catalase , oxidase , urease , citrase, MacConkey, indole , starch hydrolysis , methyl red and motility test) and their genus were identified based on Bergeys manual. In addition, the effects of antimicrobial agent and temperature on the growth of bacteria from fruit skins were investigated.

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0.3 PAPAYA 4DAYS IM30/IN30

											SUM	OVERALL
	1.00E+00	1.00E+02	1.00E+03	1.00E+04	1.00E+05	1.00E+06	1.00E+07	1.00E+08	1.00E+09	1.00E+10		
1	0	0	0	0	0	0	0	135	138	0		
	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.35E+10	1.38E+11	0.00E+00	1.52E+12	
												1.34E+12
2	0	0	0	0	0	0	0	148	101	0		
	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.48E+10	1.01E+11	0.00E+00	1.16E+12	

0.3 PAPAYA 4DAYS IM4/IN30

											SUM	OVERALL
	1.00E+00	1.00E+02	1.00E+03	1.00E+04	1.00E+05	1.00E+06	1.00E+07	1.00E+08	1.00E+09	1.00E+10		
1	0	0	0	0	0	0	0	0	0	0		
	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	
2	0	0	0	0	0	0	0	0	0	0		
	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	

0.3 PAPAYA 4DAYS IM30/IN4

											SUM	OVERALL
	1.00E+00	1.00E+02	1.00E+03	1.00E+04	1.00E+05	1.00E+06	1.00E+07	1.00E+08	1.00E+09	1.00E+10		
1	0	0	0	0	58	61	40	0	0	0		
	0.00E+00	0.00E+00	0.00E+00	0.00E+00	5.80E+06	6.10E+07	4.00E+08	0.00E+00	0.00E+00	0.00E+00	4.67E+09	
												2.92E+10
2	0	0	0	0	60	0	67	47	0	0		
	0.00E+00	0.00E+00	0.00E+00	0.00E+00	6.00E+06	0.00E+00	6.70E+08	4.70E+09	0.00E+00	0.00E+00	5.38E+10	

0.3 PAPAYA 4DAYS IM4/IN4

											SUM	OVERALL
	1.00E+00	1.00E+02	1.00E+03	1.00E+04	1.00E+05	1.00E+06	1.00E+07	1.00E+08	1.00E+09	1.00E+10		
1	0	0	0	0	0	0	0	0	0	0		
	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	
2	45	0	0	0	0	0	0	0	0	0		
	4.50E+01	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	4.50E+02	

CONDITION	CFU/ML
IM30/IN30	1.34E+12
IM4/IN30	0
IM30/IN4	2.92E+10
IM4/IN4	225

4days papa control	0.3	0.6	
IM30/IN30	4.83E+12	1.3365E+12	4.00E+11
IM4/IN30	5.05E+12	0	4.00E+11
IM30/IN4	3.51E+10	29214000000	3.30E+12
IM4/IN4	3.49E+09	225	2.56E+09

