ISOLATION AND CHARACTERIZATION OF BACTERIA FROM THE SKINS OF GUAVA AND APPLE

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ABSTRACT

In recent years, cut fruit products get a warmest hit among current community. A rapid lifestyle changes among most civilizations leads them to choose a convenient way to get balanced meal and nutrients. The control of microbial growth in cut fruits is an important aspect. This study aimed to isolate and characterize the bacteria from apple and guava fruit skins. Moreover, this study also aimed to investigate the effect of temperature and antimicrobial agent in controlling the growth of bacteria from fruit skins. Six bacteria from guava and seven bacteria from apple fruit skins were successfully isolated. These bacteria were then characterized using biochemical tests. Based on Bergey's manual, the bacteria were classified as *Staphylococcus* spp., *Proteus* spp., *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Erwinia* spp. and *Enterobacter* spp.. Two parameters, which were antimicrobial agent (XY-12) concentration and temperature, were optimized to control the growth of bacteria in the fruit skins. Results revealed that the optimum XY-12 concentration and temperature in retarding the growth of bacteria were 0.6 mL/L and 4°C respectively. A total of 99.4% of bacterial growth reduction was achieved when guava skins were treated with 0.6 mL/L of XY-12 and incubated at 4°C for 4 days in comparison with the control. In addition, a 100% of bacterial growth inhibition was observed when apple skins were treated under the same conditions. The antimicrobial assays (disc diffusion method) were also performed individually on the 13 isolated bacteria. At 0.6 mL/L of XY-12, largest zone of inhibition (2.70 cm) was observed in strain SA 4 after 24 hours of incubation followed by 2.60 cm (strain SG 5) and 2.46 cm (strain SA 2 and SA 3). Negative control (disc with distilled water) did not show any zone of inhibition.

ABSTRAK

Kebelakangan ini, produk buah-buahan yang dipotong mendapat sambutan yang memberangsangkan di kalangan masyarakat. Perubahan gaya hidup yang pesat di kalangan masyarakat kini telah mendorong mereka untuk memilih cara yang mudah untuk mendapatkan makanan dan nutrien seimbang. Pada masa kini, kawalan pertumbuhan mikrob dalam buah-buahan dipotong adalah satu aspek penting yang harus dipertimbangkan. Kajian ini bertujuan untuk memencilkan dan mengkaji ciri-ciri bakteria daripada kulit epal dan buah jambu. Selain itu, kajian ini juga bertujuan untuk mengkaji kesan suhu dan antimikrobial ejen dalam mengawal pertumbuhan bakteria pada kulit buah-buahan. Sebanyak enam bakteria daripada buah jambu dan tujuh daripada buah epal telah berjaya dipencilkan. Bakteria ini kemudiannya dianalisis dengan menggunakan ujian biokimia. Berdasarkan manual Bergey's, bakteria dikelaskan sebagai *Staphylococcus* spp., *Proteus* spp., *Micrococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Erwinia* spp. dan *Enterobacter* spp.. Dua parameter, iaitu kepekatan ejen antimikrob (XY-12) dan suhu, telah dioptimumkan untuk mengawal pertumbuhan bakteria pada kulit buah-buahan. Hasil kajian menunjukkan bahawa kepekatan XY-12 dan suhu yang optimum dalam membantutkan bakteria adalah 0.6 mL/L and 4°C. Sebanyak 99.4% kadar pengurangan pertumbuhan bakteria telah dicapai apabila kulit jambu dirawat dengan 0.6 mL/L XY-12 dan dieram pada 4°C selama 4 hari berbanding dengan kawalan. Di samping itu, 100% perencatan pertumbuhan bakteria diperhatikan apabila kulit epal telah dirawat di bawah keadaan yang sama. Ujian antimikrob dengan kaedah penyebaran cakera juga telah dijalankan bagi 13 bakteria yang telah diperincilkan. Pada 0.6 mL/L XY-12, zon terbesar perencatan (2.70 cm) diperhatikan pada bakteria SA 4 selepas 24 jam masa inkubasi diikuti 2.60 cm (bakteria SG 5) and 2.46 cm (bakteria SA 2 and SA 3). Kawalan negatif yang dicelup dengan air suling tidak menunjukkan apa-apa zon perencatan.

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LIST OF ABBREVIATIONS

∞	Infinity
%	Percentage
°C	Degree Centigrade Celsius
$US	United States *Dollars*
16S rRNA	16 small subunit of ribosomal Ribonucleic acid
bp	Base pairs
cm	Centimeters
CFU	Colony forming unit
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
Et al	And others
ETBR	Ethidium bromide
FDA	Food and Drug Administration
g / mg / µg	Gram / Milligram / Microgram
h	Hour
H_2O_2	Hydrogen Peroxide
H_2O	Water
IFPA	International Fresh-cut Produce Association
IR	Infrared radiation
kPa	Kilo Pascal
kGy	KiloGray
L / ml / µL	Liter / Milliliter / Microliter
min	Minutes
M / mM / µM	Molar / Millimolar / Micromolar
MAP	Modified Atmosphere Packaging
MH	Muller Hinton
$MgCl_2$	*Magnesium Chloride*
MPV	Minimally processed vegetables
NA	Nutrient Agar
NB	Nutrient Broth
OD	Optical Density
PCR	Polymerase Chain Reaction
RM	Ringgit Malaysia
rpm	Revolutions per minute
Sec	Seconds
sp	Species
UV	Ultraviolet
V	Voltage
v/v	Volume per volume

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Fresh-cut fruits and minimally processed vegetables (MPV) are categorized as high nutrient rich food products which meet the demand of modern customers who have less time for meal preparation due to busy daily life. In fresh-cut products have been received an overwhelming response from customers due to their significant awareness to take up fruits and vegetables as a fibre source in their daily diet for health benefits (Ragaert *et al*., 2004). This event has caused the fresh-cut fruits and minimally processed vegetables (MPV) industries grow dramatically.

The primary criteria of fresh-cut products rely on their safety, nutrition content, freshness, texture and sensory quality. Maintaining the freshness and increasing the shelf-life of the products is one of the important aspects. However, the critical control area faced by the cultivars and fresh-cut fruit manufacturer is the quality spoilage due to physical injuries. The physical injuries caused from pre-harvest, harvest, post-harvest and processing will raise the respiration rate and stimulate intercellular biochemical reactions which results in degradation of texture, colour and causes microbial spoilage in fresh-cut products.

Microbial spoilage is a major challenge faced in fresh-cut industry in order to maintain nutritional composition and extend the shelf-life of the products. It also have been highlighted, due to high public concern in the food safety that associated with foodborne illness outbreak caused by microorganisms, fungal infection and pathogenic viral (Beuchat *et al*., 2002; Abadias *et al*., 2008). Based on survey done in United States, each year around 20% of processed cut-fruits are lost due to microbial spoilage (Barth *et al*., 2009). Basically, fruits provide a suitable growth environment for the bacteria, fungi and yeast (Tournas *et al*., 2006). In natural, fruits are rich in carbohydrate and sugar which serves as carbon source for the growth and multiplication of microorganisms (Naeem *et al*., 2012).

According to literatures, the most bacteria and fungi, which cause microbial spoilage on fruits, are initially soil inhabitant that introduced on the surface of the whole fruit. The surface of the fruit contains diverse community of microbes that also deposited during harvest, storage and transportation process (Barth *et al*., 2009; Mukhtar *et al*., 2010; Juhnevica *et al*., 2011). Those microorganisms can be introduced to the cut fruit products through manufacturing stages including harvesting, peeling, washing, packaging and distribution (Daniyan *et al*., 2011). So, it is important to gather information about those organisms that potentially associated with food spoilage and foodborne illness.

1.2 Problem Statement or Significance of Research

Recently, the distribution of fresh cut fruits and vegetable salads has reported an encouraging growth among local retailers and in international market. At the same time, this scenario has contributed to numerous food poisoning outbreaks as the fresh cut fruits involves less processing steps (Beuchat *et al*., 2002). Isolation and characterization of the bacterial diversity from fruit surface and the effects of antimicrobial agents in retarding the growth

of these bacteria are the important aspects to be investigated in order to improve the antimicrobial steps eventually minimizing the potential fruit spoilage and food poisoning events.

1.3 Research Objectives

The objectives of this study are:

i. To isolate bacteria from apple and guava fruit skins.
ii. To characterize the bacteria using Biochemical tests
iii. To investigate the effect of chlorine-based antimicrobial agent and temperature in inhibiting the growth of the bacteria.

1.4 Scope of Study

This research was focused on isolation and characterization of bacteria that found on the surface of guava and apple skins. The isolated bacteria were characterized based on their activity on different biochemical tests and gram staining morphology. The effect of chlorine-based antimicrobial agent (XY-12) and temperature in retarding bacterial growth were investigated by measuring the bacterial population on the skins of guava and apple after exposing them with XY-12 at different temperature.

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