IDENTIFICATION AND CHARACTERIZATION OF BACTERIA FROM THE SKIN OF JACKFRUIT

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To my beloved family

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ABSTRACT

Fresh-cut fruits industry is developing extensively in this global era due to busy lifestyle. However, the fresh-cut processing could be contaminated by microorganisms; resulting in food poisoning outbreaks. This study presented about the characterization and inhibition of bacteria isolated from the fresh skin of jackfruit. The isolation of single colonies was done by spread plate and streak plate method. The isolates were identified by 16S rRNA analysis. Gram staining, growth profiling and 15 biochemical tests were carried out to characterize each isolates. Lastly, the isolates were treated with various antimicrobial agents by disc agar diffusion technique and the fresh skin of jackfruit was treated with antimicrobial agent, XY-12 with different conditions by spread plate technique to study the effects of antimicrobial agents on microbial growth. Experimental results demonstrated that four types of bacterial single colonies were isolated with different morphology and designated as CE1, CE2, CE3 and CE4. The 16S rRNA analysis showed that the isolates of CE1 and CE4 were *Bacillus* sp. strain CY-b33. Isolates of CE2 and CE3 were identified as *Bacillus pumilus* strain SBTBP-008 and *Bacillus thuringiensis* strain EA26.1 respectively. All the isolates were Gram positive and in rod shaped. The growth kinetics of CE3 was highest (0.2280 h^{-1}) compared to CE1 (0.1317 h^{-1}) which was the lowest. Bacteria CE1 and CE2 have the highest sensitivity against XY-12 (0.6 mL/L) and Kanamycin Sulfate respectively. Bacteria CE3 and CE4 have the highest sensitivity against Ampicillin Trihydrate and Tetracycline Hydrochloride respectively. The fresh skin of jackfruit treated with XY-12 (0.6 mL/L) and incubated at 4°C revealed high efficiency in microbial reduction at day 2 (100%) and 4 (99.72%).

KEYWORDS: 16S rRNA analysis, isolates, Gram staining, XY-12, *Bacillus* sp., *Bacillus pumilus, Bacillus thuringiensis.*

ABSTRAK

Industri buah-buahan hirisan segar telah membangun maju dalam era global ini disebabkan oleh gaya hidup yang sibuk. Walau bagaimanapun, pemprosesan hirisan segar boleh menyebabkan kerosakan oleh mikroorganisma; mengakibatkan wabak keracunan. Kajian ini membentangkan tentang pengenalan, pencirian dan seterusnya perencatan bakteria yang diasingkan daripada kulit segar nangka. Pengasingan bakteria koloni tunggal telah dilakukan dengan kaedah piring sebaran dan piring garis jalur. Bakteria yang diasingkan dikenal pasti melalui analisis 16S rRNA. Pewarnaan Gram, profil pertumbuhan dan 15 ujian biokimia telah dijalankan untuk mencirikan setiap koloni. Kesemua bakteria telah dirawat dengan pelbagai agen antimikrob menggunakan teknik penyebaran disk agar dan kulit segar nangka telah dirawat dengan agen antimikrob, XY-12 dengan syarat-syarat yang berbeza melalui teknik piring sebaran untuk mengkaji kesan agen antimikrob pada pertumbuhan mikroorganisma. Keputusan eksperimen menunjukkan bahawa empat jenis koloni bakteria tunggal telah diasingkan dengan morfologi yang berbeza dan ditetapkan sebagai CE1, CE2, CE3 dan CE4. Keputusan 16S rRNA menunjukkan bahawa bakteria CE1 dan CE4 adalah *Bacillus* sp. jenis CY-b33. Manakala, CE2 adalah *Bacillus pumilus* jenis SBTBP-008 dan CE3 menunjukkan *Bacillus thuringiensis* jenis EA26.1. Semua bakteria yang diperoleh adalah Gram positif dan berbentuk rod. Kinetik pertumbuhan CE3 adalah tertinggi (0.2280 h^{-1}) berbanding CE1 (0.1317 h^{-1}) yang paling rendah. Bakteria CE1 dan CE2, masing-masing mempunyai sensitiviti yang tertinggi terhadap XY-12 (0.6 mL/L) dan Kanamycin Sulfat. CE3 mempunyai sensitiviti yang tertinggi terhadap Ampicillin Trihydrate manakala CE3 terhadap Tetracycline Hydrochloride. Kulit segar nangka dirawat dengan XY-12 (0.6 mL/L) dan disimpan pada suhu 4°C mendedahkan kecekapan tinggi dalam pengurangan mikroorganisma pada hari ke-2 (100%) dan ke-4 (99.72%).

KATA KUNCI: analisis 16S rRNA, koloni tunggal, pewarnaan Gram, XY-12, *Bacillus sp, Bacillus pumilus, Bacillus thuringiensis.*

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LIST OF ABBREVIATIONS

CFU/mL	-	Colony Forming Units per millilitre
kb	-	kilobytes
PCR	-	Polymerase Chain Reaction
16S rRNA	-	16 Subunit Ribosomal Ribonucleic Acid
bp	-	base pair
ng/µL	-	Nanogram per microlitre
°C	-	degree Celsius
µ	-	micro (unit of growth rate)
g	-	unit of generation time
h^{-1}	-	per hour
h	-	hour
nm	-	nanometre
mL/L	-	mililitre per litre
ppm	-	parts per milion

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Nowadays, the markets of fresh-cut fruits have shown a marked upward trend in Malaysia and also in international markets. Occasionally, fresh fruits can become contaminated with harmful microorganisms (Peggy VL *et al*., 2001). The fresh fruits are exposed to microbial contamination through a variety of sources. Environmental contamination such as water quality, soil fertility management, equipment sanitation and many other factors contribute to the risk of microbial contamination. In addition, the microbial contamination with fresh produce could be associated with human or animal feces (Pradnya and Sonali, 2008).

According to survey, the total production of the fruit in the world is around $27x10^6$ ton per year (Hathaitip *et al*., 2013). A survey in United Kingdom shows that 15-20% of fresh-cut fruits are lost each year due to the microbial contamination (Bond *et al*., 2013). Recently, the Federal Food and Drug Administration from United States has reported the outbreak of *listeria* infection from cantaloupe which has killed 33 people (Reuters, 2013). Listeriosis, a serious bacterial infection usually caused by eating food or fruits contaminated with the *Listeria monocytogenes*, is an important public health problem in the United States (Centers for Disease Control and Prevention, 2013).

Jackfruit is rich in vitamins A, C and B-complex with abundant of fiber, minerals and energy. Nowadays, people who live a busy lifestyle are interested in

fresh-cut fruits, which jackfruit is one of them. During the cutting process of fresh jackfruit, the microorganisms on the surface of a skin of jackfruit may be transferred into the flesh of fruit. These bacteria utilize the host via extracellular lytic enzymes that hydrolyzed these polymers to release water and the other intracellular constituents for use as nutrients for their growth (Margaret B *et al.*, 2009). As a result, the quality of the fruit is affected. To prevent the microbial spoilage, effective preventive measures should be taken to prevent microbial spoilage on fresh-cut fruits.

In this project, bacteria from the skin of jackfruit were isolated and the diversity of the bacterial population were studied. These bacteria were morphological and biochemically characterized. The bacteria were identified using 16S rRNA gene analysis. The effectiveness of XY-12, a commercial available antimicrobial agent, was used to test its efficiency in retarding the growth of these bacteria.

1.2 Problem Statement/ Significance of Research

The processing steps of cleaning, trimming, coring, slicing, shredding, washing, centrifugal drying and packaging for production of fresh-cut fruits have been well developed (Chung CC *et al.*, 2011). However, the processes are sometimes not hygienic enough and could cause a numerous fruits contamination and poisoning outbreaks. The presence of bacteria on the cut-fruits could proliferate and thus decrease the shelf-life of the products. Attempt to isolate and characterize the bacteria from the skin of the jackfruit, and the studies of the effect of antibacterial agent on these bacteria are crucial because these findings provide useful information on the improvement of antimicrobial steps to produce a good quality of fresh cut-fruits with better shelf-life.

1.3 Research Objectives

Followings are the objectives of this research:

1.3.1 To isolate bacteria from the skin of jackfruit.

1.3.2 To identify the bacteria using 16S rRNA analysis.

1.3.3 To characterize bacteria using biochemical tests and antimicrobial agents.

1.4 Scope of Research

In this study, the bacterial diversity on the skin of jackfruit was investigated. The bacteria were isolated by using serial dilution and spread plate technique. Then, these bacteria were indentified based on 16S rRNA analysis. The bacteria were morphologically and biochemically characterized. The effect of chlorine based antimicrobial agent (XY-12) and temperature in inhibiting the growth of bacteria from jackfruit skin were investigated by measuring the population of bacteria on the skin after being treated with XY-12 at different temperature.

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