EFFECT OF ANTIMICROBIAL AGENT TOWARD BACTERIA FROM SKIN OF CANTALOUPE FRUIT

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ABSTRACT

Fresh cut-fruits produce is one of the quickest developing food industries ever. Food poisoning has been connected with the utilization of poor quality of fruits. The present study was conducted to isolate bacteria from cantaloupe skin, characterize and identify the bacteria from cantaloupe fruit skins using biochemical tests and 16S rRNA gene analysis and to investigate the effect of antimicrobial agents on the growth of bacteria and all of these objective were successfully achieved. Four unique bacteria based on different morphology were isolated by using spread plate method. A total of ten biochemical tests were performed. For bacterial identification, the PCR products of the 16S rRNA gene were amplified, purified and sent for sequencing. Bacterium CG1, CG2, CG3 and CG4 bacteria were identified as *Exigubactirum* sp CG1, *Exigubactirum* sp. CG2, *Pseudomonas* sp. CG3, and *Microbacterium* sp. CG4. The XY-12 antimicrobial agent with two different concentrations (0.6 mL/ L and 0.3 mL/ L) were tested to inhibit the growth of bacteria in the fruit skins at two different temperatures (4 $^{\circ}$C and 30 $^{\circ}$C). It was observed that 100% of microbial reduction was achieved when the sample of fresh cantaloupe skins were immersed in 0.6 mL/ L of XY-12 and incubated at 4 $^{\circ}$C incubated for 2 days. For 4 days of incubation under the same condition (0.6 mL/Lof XY-12; 4 $^{\circ}$C), 99.98% of microbial reduction were observed. All isolated bacteria were also screened against four different antibiotics using disc-diffusion assay and discs impregnated with distilled water were used as negative control. The result of Kanamycin sulfate (1 mg/mL) found to be the most effective against bacterial strain of CG2 with the average zone of inhibition of 29 mm. No zones of inhibition were observed with negative control discs.

ABSTRAK

Buah-buahan potong segar adalah salah satu industri makanan yang paling cepat membangun. Keracunan makanan adalah berkaitan dengan penggunaan buah-buahan yang berkualiti rendah. Kajian ini telah dijalankan untuk: i) mengasingkan bakteria daripada kulit tembikai ‘cantaloupe’; ii) mencirikan dan mengenal pasti bakteria daripada kulit buah tembikai‘cantaloupe’ menggunakan ujian biokimia dan analisis gen 16S rRNA dan iii) untuk mengkaji kesan agen antimikrob terhadap pertumbuhan bakteria dan semua matlamat ini telah berjaya dicapai. Empat bakteria unik berdasarkan morfologi yang berbeza telah diasingkan dengan menggunakan kaedah plat penyebaran. Sebanyak sepuluh ujian biokimia telah dijalankan. Untuk mengenal pasti bakteria, produk PCR daripada gen 16S rRNA telah diamplifikasi, dibersihkan dan dihantar untuk penjujukan. Bakteria CG1, CG2, CG3 dan CG4 telah dikenal pasti sebagai *Exigubactirum* sp. CG1, *Exigubactirum* sp. CG2, *Pseudomonas* sp. CG3, dan *Microbacterium* sp. CG4. Anti mikrob XY-12 dengan dua kepekatan yang berbeza (0.6 mL / L dan 0.3 mL / L) telah diuji untuk menghalang pertumbuhan bakteria dalam kulit buah pada dua suhu yang berbeza (4 °C dan 30 °C). Berdasarkan permerhatian didapati bahawa 100% pengurangan mikrob dicapai apabila sampel kulit tembikai segar direndam dalam 0.6 mL / L XY-12 dan dieram pada 4 °C selama 2 hari. Selama 4 hari pengeraman di bawah keadaan yang sama (0.6 mL / L XY-12; 4 °C), sebanyak 99.98% daripada pengurangan mikrob diperhatikan. Semua bakteria yang diasingkan juga disaring dengan menggunakan empat antibiotik yang berbeza menggunakan rentasan ‘disc’ penyebaran dan ‘disc’ yang dimuatkan dengan air suling digunakan sebagai kawalan negatif. Hasil Kanamycin sulfat (1 mg/mL) didapati paling berkesan terhadap strain bakteria CG2 dengan zon purata perencatan sebanyak 29mm. Tiada zon perencatan diperhatikan pada kawalan negatif.

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LIST OF SYMBOLS AND ABBREVIATIONS

MRSA	-	Methiciline resistance *s.aureus*
TSS	-	Toxic sick syndrome
BLAST	-	Basic Local Alignment Tool
O-F	-	Oxidative fermantive
cfu/g	-	Colony Forming Unit
Cfu	-	Colony Forming Unit
CaCl2	-	Calcium Chloride
°C	-	Degree Celcius
DNA	-	Deoxyribonucleic Acids
EDTA	-	Ethylene Diamine Tetraacetic Acid
Td	-	Mass doubling time
FFV	-	Standard for fresh cut fruit
g	-	Gram
Gb	-	GenBank
g/ml	-	Gram Per Millilitre
Hr	-	Hour
$	-	dollar
H_2O	-	Water
Bp	-	Base Pairs

Kbp	-	Kilo base pairs
L	-	Litre
Min	-	Minute
mg/ml	-	Miligram per millilitre
$MgSO_4$	-	Magnesium sulphate
Ml	-	Mililitres
mM	-	Milimolar
Mm	-	Milimetre
M	-	Molar
Ng	-	Nanogram
NCBI	-	*National Center for Biotechnology Information*
NA	-	Nutrient agar
NB	-	Nutrient Broth
NaCl	-	Sodium chloride
NaOH	-	Sodium hydroxide
ng/µL	-	Nanogram per microliter.
%	-	Percent
OD	-	Optical density
PCR	-	Polymerase Chain Reaction
rpm	-	Revolutions per minute
PME	-	Pectin methylesteras
UV	-	Ultraviolet
µL	-	Microlitre
M	-	Specific growth rate
v/v	-	Volume per volume

w/v	-	Weight per volume
PG	-	polygalacturonase
EFSA	-	European food safety agency

CHAPTER 1

INTRODUCTION

1.1 Background of The Study

Fresh cut produce are known as fruits, which are pleed, cut, or prepared to give suitable prepared to consume or prepared to cook portion. It is one of the quickest developing comfort nourishment businesses ever. The fundamental issue that makes cut-fruit grown from the ground a very perishable item is the microbial development brought about by the amassing of spilled juice rich in supplements at the base of containers (Brecht, 2006).

Cantaloupe fruits are currently acknowledged worldwide and accessible year-round all through the United States. Conversely, this item is exceptionally perishable (O'connoret*et al.*, 1994; Portela and Cantwell, 1999) and in the same manner as different melon fruits, has shown to be connected with epidemics of disease-associated Salmonellosis (CDC, 1991 and Tamplin, 1997). Continued existence and development of *Salmonella* sp. also *E. coli* O157:H7 on skin cantaloupe together with tissue have been described and documented (Golden *et al*,. 1993). In spite of the operations, for example, peeling, slicing, or cutting incredibly build tissue harm of cantaloupe fruits, bringing about the discharge of intracellular fluids, and hence

increment of microbial development, which is the primary deteriorative and well being issue included in the new cut-fruit industry (Brecht, 2006).

As of late, the federal ministry of food and drug administration from the United State of America has found the epidemics of *Listeria* contamination generally brought about by consuming some nourishment or products of the soil infected with *Listeria monocytogenes*, is a paramount general wellbeing issue in United State of America (Center of ailments control and avoidance, 2013). On the other hand, in the United States, *Salmonella* marked out to Mexican rock melons caused epidemics in 2000, 2001 and 2002. The examination was tedious and created a decrease in California cantaloupe transactions after the Mexican developing season.

The primary method to minimize bacterial debasement in foods grown from the ground (fresh cut fruits and vegetables) is through temperature control. Despite the fact that by controlling the temperature could be a methodology to minimize the development of microorganisms, mutual treatments are needed. This is due to the reason that *Salmonella hadar* could strive well and multiply on ice-cold tattered slice products of the soil earlier to deterioration (Piagentini *et al.,* 1997). While development of a few pathogenic organisms could be possibly hindered by freezing temperatures, continued existence can be improved under specific environments. Case in point, *Escherichia coli O157:H7* and *Salmonella* species can endure for a long time period in fruit juices under ice-cool condition than at room temperatures (Parish *et al.,* 1997 and Zhao *et al*., 1993).

Chlorinated compounds have been utilized for hygienic purposes as a part of vegetable foods and other nourishment transforming for a few decades and is maybe the most generally utilized sanitizer as a part of the sustenance business in food processing industry (Walker and Lagrange, 1991). At the nourishment administration together with domestic stage, chlorine becomes a suitable and cheap sanitizer for controlling foodborne pathogenic organisms.

1.2 Problem Statement/Significance of Study

Fresh-cut fruit industry is among the fastest emerging convenience food industry in history. Due to reason being that fresh-cuts are perishable items, they can easily be contaminated by microbes. In some cases, the microorganisms cause food poisoning epidemics or outbreaks. This study investigated the bacterial diversity on the skin of Cantaloupe melon. The effect of antimicrobial agents was also be evaluated on their efficiency in inhibiting the growth of these microbes.

1.3 Specific Objective of the Study

1) To isolate and identify bacteria from the skin of cantaloupe melon.
2) To characterize the bacteria using biochemical tests.
3) To investigate the effect of antimicrobial agents on the growth of isolated bacteria.

1.4 Scope of the Study

For this study, bacteria were isolated from the skin of fresh Cantaloupe melon by employing serial dilution and spread plate techniques. Purified colonies of bacteria were identified through 16S rRNA analysis. Additionally, the isolated bacteria were characterized chemically by various biochemical tests, and finally the effects of antimicrobial agents in retarding the bacterial growth were examined.

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