UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND SCREENING OF ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA ISOLATED FROM DAIRY PRODUCTS

SITI NUR ALIA RAMLI

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "Identification and screening of antimicrobial activity of lactic acid bacteria isolated from the dairy products" telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh SITI NUR ALIA BINTI RAMLI (162568) sebagai syarat untuk kursus BMY 4999 projek.

Disahkan oleh:

…….. Tarikh: …….

Dr. Wan Zuhainis Saad

Penyelia

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

…… Tarikh: …….

Prof. Madya Dr. Muhajir Hamid

Ketua

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

ABSTRACT

This study was conducted to screen for the antimicrobial activity of isolated LAB from dairy product and to identify the genera of the LAB. Four isolates of unknown bacteria were obtained from Mycology Laboratory at Biotech 2 with the permission from the supervisor. All the unknown bacteria were isolated from 4 diferrent resources of dairy product. Among four isolates, only one showed the clear zone in the screening of antimicrobial activity against *Escherichia coli* and *Listeria monocytogenes*. The isolate was labelled as sample 1, it showed rod shape under the microscope and was revealed as the gram positive bacteria due its appearace in purple colour on gram staining test. Presumption test was done on sample 1 with a few series of biochemical test and it was identified as *Lactobacillus* sp. The LAB was tested using critical dilution method to determine the quantitatively amount of the antimicrobial activity on *E. coli and L. monocytogenes*. Regarding to this finding, it can be further commercialized on fighting the food disease associated by food borne pathogen and and may involve in the food preservative method.

ABSTRAK

Projek ini telah dilakukan untuk saringan aktiviti antimikrob terhadap bakteria asid laktik daripada produk tenusu dan untuk mengenal pasti jenis bakteria asid laktik. Empat jenis bakteria yang tidak diketahui telah diperolehi dari Makmal Mikologi di Biotech 2 dengan kebenaran daripada penyelia. Semua bakteria tidak diketahui dimaklumkan telah diperoleh daripada 4 sumber tenusu yang berbeza. Antara empat bakteria yang diambil hanya satu bakteria yang tetap zon jelas dalam aktiviti antimikrob terhadap E. coli dan L. monocytogenes. Bakteria yang telah dilabelkan sebagai sample 1 ini menunjukkan bentuk rod di bawah mikroskop dan dinyatakan sebagai bakteria gram positif kerana kelihatan warna ungu pada ujian gram pewarnaan. Ujian anggapan dilakukan pada sample 1 dengan beberapa siri ujian biokimia dan telah dikenalpasti sebagai sebagai Lactobacillus sp. LAB telah menjalani kaedah pencairan kritikal untuk menentukan jumlah yang kuantitatif aktiviti antimikrob pada E.coli dan L. monocytogenes. Berkenaan dengan penemuan ini, ia mungkin boleh dikomersialkan pada masa hadapan untuk melawan penyakit makanan yang berkaitan dengan patogen bawaan dan dalam kaedah pengawetan makanan.

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ABBREVIATIONS

Abbreviations for the names of units used in the text:

AU	Arbitrary unit
LAB	Lactic acid bacteria
µl	micro litre
g	gram
mg	miligram
µg	microgram
NB	nutrient broth
OD	optical density
nm	nanometre
l	litre
ml	millilitre
EHEC	Enteromorrhagic *Escherichia coli*
Da	Dalton
Cfu	colony forming unit
^{o}C	Degree Celsius
nm	nanometers
GC content	guanine-cytosine content

CHAPTER 1

INTRODUCTION

Food borne disease still linger as one of the greatest public health problems in Malaysia and other developing country. Ingestion of food stuff contaminated with microorganisms or chemical will lead to the outbreak of food born disease such as cholera, typhoid fever, hepatitis A, dysentery and food poisoning. Most of the food borne illnesses is excruciating, meaning they happen suddenly and most of patient recover on their own without treat but sometimes food borne disease may lead to more serious complication such as dehydration and will become worst which can cause chronic health problem and even can cause death. Symptoms of food borne disease are dependent on the type of microorganism or chemical present but most of them share common symptoms including vomiting, diarrhea or bloody diarrhea, abdominal pain, fever, chills and all these symptoms can extent from lenient to grievous and can last a few hours to several days.

E. coli, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* always become the popular microorganisms that can cause the food borne disease (Cappucino and Sherman, 2005). All these pathogen can infect human gastrointestinal tract and some of them can dangerously affect the nervous system which show some symptoms such as headache, tingling, weakness, numbness of skin, blurred vision and dizziness. If we want to prevent the food poisoning from happen, we have to deal with the prevention from getting infected by the pathogen and we have to think about how to kill microorganism or suppressing their growth when if we already got infected. Table 1 shows the type of food borne pathogen,

characteristic and example for each pathogen that always being isolated from the person who got food poisoning.

Table 1 : Food borne pathogen (Sharifa et al., 2013)

Type of pathogens	Characteristics	Example
Bacteria	In environment or animal resevoir and multiply in or on food	*Campylobacter* and *Salmonella*
Virus	Reproduce only in within living cell and remain infectious in food	Hepatitis A and Norwalk virus
Parasite	Reproduce within the host cells and cannot multiply on food	*Gardia lamblia*
Toxin	Produce by some bacteria or chemical	*Bacillus cereus, Staphylococcus aureus, E.coli* and *Clostridium botulinum*

Most of parents prefer to give dairy product to their children to prevent them from get infected by the pathogen. They believe the content of the product especially lactic acid bacteria that present in the milk can help to protect their children from the pathogen. From here lactic acid bacteria isolated from dairy product have received attention as a potential microorganism that can help us to protect our health. Japan 1930, Dr. Minoru Shirota, who was conducting research in a Microbiology Lab the Medical Faculty of Kyoto University, became the first person in the world that succeed in culturing a strain of lactic acid bacteria which give beneficial to human health especially to prevent from being infected by food borne pathogen. He wanted

to develop a stronger strain of lactic acid bacteria which would destroy the harmful bacteria living in the intestinal because the pathogen can cause the irritation of gastro intestinal tract.

Lactic acid bacteria are gram positive bacteria that able to ferment glucose primarily into lactic acid. Beside lactic acid, it also produced acetic acid, hydrogen peroxide, carbon dioxide and bacteriocin which are useful in inhibiting food borne pathogen (Senthikumar et al., 2012). The low external pH of the pathogens surrounding caused the acidification of pathogens' cell cytoplasm and inhibit the growth of the pathogens. Normally lactic acid bacteria can be found in the diary product. Few of the lactic acid bacteria are pathogenic to animals especially some members of the genus *Streptococcus.* Many genera of bacteria produce lactate as a primary or secondary end product of fermentation but term lactic acid bacteria is well known reserved for genera of *Lactobacillus,* which includes *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus and Streptococcus* (Fujitoshi et al., 2005). Therefore, the aim of this final year project was to identify lactic acid bacteria from the dairy product in addition to detect the antimicrobial effects of isolated lactic acid bacteria against a few types of food borne pathogen.

Hypothesis

1. Lactic acid bacteria which isolated from dairy product may have antimicrobial activity.

Objectives

1. To identify the lactic acid bacteria that has the antimicrobial activity against pathogenic bacteria.
2. To measure the amount of the antimicrobial activity
3. To identify the species of the lactic acid bacteria that has the antimicrobial activity.

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