

UNIVERSITI TEKNOLOGI MARA

**CHARACTERIZATION OF NOVEL
PROBIOTIC LACTIC ACID
BACTERIA ISOLATED FROM
MALAYSIAN FERMENTED SHRIMP
(*PEKASAM SENEK*) WITH
CHOLESTEROL-LOWERING
EFFECT**

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ABSTRACT

Lactic acid bacteria (LAB) from fermented foods are proven to be able to hinder the growth and activities of some foodborne pathogens. This study aims to isolate and characterize the potential LAB isolated from Malaysian fermented shrimps, *Pekasam senek*. Twenty potential LAB isolates were assessed for their antagonistic activities against *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Shigella sonnei* ATCC 11778 via the agar spot antimicrobial assay. The antibiotic susceptibility patterns of the isolates to six types of antibiotics were assessed through the disc diffusion method. The potential isolates then were proceeded with a subsequent assay, which is the hemolytic assay where the fresh bacterial cultures were streaked on a fresh blood agar plate containing 5% (w/v) sheep blood, and incubated at 37°C for 24-48 hours to examined for signs of β -hemolysis, α -hemolysis and γ -hemolysis. All selected isolates were further evaluated for their acid and bile salt tolerance. Moreover, the potential LAB isolates were characterized using 16S rRNA genes and were continued with evaluating cholesterol-lowering activity. In antagonistic evaluation, the results showed that all LAB isolates could inhibit the growth of indicator bacteria with the highest inhibition zone (31 mm) produced by PS26 against *E. coli*. Furthermore, nineteen (19) isolates were susceptible to ampicillin, bacitracin, chloramphenicol, and erythromycin whilst resistant to streptomycin. Twelve (12) potential isolates showed negative hemolytic activity and were preceded for evaluating acid and bile salt tolerance. The potential isolates showed satisfactory tolerance in harsh acidic condition of pH 3.0; ten potential isolates (PS13, PS14, PS16, PS17, PS20, PS24, PS26, PS27, PS31, PS33) exhibit over 90% of tolerance, isolate PS22 with 85.37% of tolerance, and isolate PS15 with 75.73% of acid tolerance. While in bile salts tolerance, nine potential isolates (PS13, PS14, PS15, PS16, PS17, PS24, PS27, PS31, and PS33) survived with 50% to 95% survivability rate after 6 hr of exposure. Despite showing a low tolerance to bile salt compared to the control, isolates PS20, PS22, and PS27 were still growing. The strains were identified as *Lactiplantibacillus plantarum* (PS13, PS15, PS17, PS20, PS24, and PS31), *Bacillus cereus* (PS14, and PS16) and *Bacillus* spp. (PS22, PS26, and PS27) using 16S rRNA gene sequencing. Lastly, PS24 and PS31 showed the highest cholesterol lowering with 80.50% and 80.19% respectively. While PS13, PS14, PS16, PS17, PS20, PS26, PS27, and PS33 showed cholesterol lowering above 70%, whilst PS22 showed only 50.55% reduction. This study indicates that LAB isolated from *Pekasam senek* had significant antagonism ability against the tested indicator bacteria with negative hemolysis. Meanwhile, the antibiotic susceptibility, acid and bile salt tolerance patterns of the isolates were varied depending on different types of antibiotics, pH, and bile salt concentration. These results proved that the isolates have good properties as probiotics with positive cholesterol lowering activity which can promote optimal health and minimize the illness risk.

Keyword: Lactic acid bacteria (LAB), probiotic, fermented food, *Pekasam senek*, cholesterol lowering

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TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF SYMBOLS	xi
LIST OF ABBREVIATIONS	xii
CHAPTER 1 INTRODUCTION	1
1.1 Research Background	1
1.2 Problem Statement	3
1.4 Research Objectives	4
1.5 Significance of Study	4
CHAPTER 2 LITERATURE REVIEW	5
2.1 Introduction	5
2.1.1 Probiotic: A Brief History	5
2.2 Properties of Probiotics	8
2.2.1 Positive Antagonism Activity	9
2.2.2 Antibiotic Susceptibility	10
2.2.3 Hemolysis Negative	11
2.2.4 Acid Tolerance	12
2.2.5 Bile Salt Tolerance	13
2.3 Health Benefits of Probiotics	14
2.3.1 Cardiovascular Disease and Hypercholesterolemia	15
2.3.2 Probiotics with Cholesterol-Lowering Effect	17
2.4 Food Fermentation	20

CHAPTER 1

INTRODUCTION

1.1 Research Background

There are many records of complications in human health due to diet, stress, and modern medical practices (antibiotics and radiotherapy) (Sitdhipol et al., 2023). Some common human disorders reported include gastrointestinal infection and high cholesterol levels. These two diseases are prevalent and have been among the most severe health issues for many years (Huang et al., 2019). Therefore, functional foods and nutraceuticals have recently received considerable attention for their benefits to human health.

Several recent studies have demonstrated the importance of probiotics as a natural, healthy, and efficient barrier against microbial infections in the guts system of humans and animals (Shehata et al., 2016). For several decades, using probiotics to foster ideal health and minimize illness threats has been a widely discussed topic (Ahmad et al., 2018). Probiotics are beneficial bacteria that have beneficial health effects on the host, including: (1) helping prevent chronic diseases, (2) preserving the intestinal microflora balance, (3) inhibiting the growth of pathogenic bacteria, (4) enhancing digestion, (5) boosting resistance to infection, (6) improving immune modulation and intestinal function, (7) suppressing diabetes, obesity, and heart disease, (8) removing carcinogens from the intestinal tract, (9) reducing allergic reactions, (10) reducing cholesterol content, and (11) improving lactose intolerance (Ahmad et al., 2018; Sitdhipol et al., 2023).

Probiotics also have health benefits and antimicrobial effects on pathogenic bacteria in the gut of individuals (Al-Khalaifah, 2018). La Fata et al. (2018) claimed that consuming probiotics could prevent gastrointestinal discomfort and enhance the immune system. *Bifidobacterium* plays a vital role in enriching human health and inhibiting the activity of *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Bacillus cereus*, and *Clostridium perfringens*, which can cause colonic disease and colon cancer (Sitdhipol et al., 2023).