การศึกษาคุณลักษณะของโพรไบโอติกแบคทีเรียกรดแลกติกที่มีศักยภาพลดคลอเลสเตอรอลในหลอดทดลอง และ สัตว์ทดลอง และ การประยุกต์ใช้โพรไบโอติกแบคทีเรียกรดแลกติกในผลิตภัณฑ์นม

CHARACTERIZATION OF PROBIOTIC LACTIC ACID BACTERIA WITH CHOLESTEROL-LOWERING POTENTIAL *IN VITRO* AND *IN VIVO* AND THEIR APPLICATION IN DAIRY

PREEYARACH WHISETKHAN

GRADUATE SCHOOL Srinakharinwirot University

2018

การศึกษาคุณลักษณะของโพรไบโอติกแบคทีเรียกรดแลกติกที่มีศักยภาพลดคลอเลสเตอรอลในหลอดทดลอง และ สัตว์ทดลอง และ การประยุกต์ใช้โพรไบโอติกแบคทีเรียกรดแลกติกในผลิตภัณฑ์นม

ปรียารัช วิเศษขันธุ์

ปริญญานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตร
วิทยาศาสตรมหาบัณฑิต สาขาวิชาชีวภาพการแพทย์
คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
ปีการศึกษา 2561

CHARACTERIZATION OF PROBIOTIC LACTIC ACID BACTERIA WITH CHOLESTEROL-LOWERING POTENTIAL *IN VITRO* AND *IN VIVO* AND THEIR APPLICATION IN DAIRY PRODUCT

PREEYARACH WHISETKHAN

A Thesis Submitted in partial Fulfillment of Requirements

for MASTER OF SCIENCE (Biomedical Sciences)

Faculty of Medicine Srinakharinwirot University

2018

THE THESIS TITLED

CHARACTERIZATION OF PROBIOTIC LACTIC ACID BACTERIA WITH CHOLESTEROL-LOWERING POTENTIAL *IN VITRO* AND *IN VIVO* AND THEIR APPLICATION IN DAIRY PRODUCT

BY

PREEYARACH WHISETKHAN

HAS BEEN APPROVED BY THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTER OF SCIENCE IN BIOMEDICAL SCIENCES AT SRINAKHARINWIROT UNIVERSITY

..... Dean of Graduate School

(Assoc. Prof. Dr. Chatchai Ekpanyaskul, MD.)

.....

ORAL DEFENSE COMMITTEE

..... Major-advisor

(Asst. Prof.Malai Taweechotipatr, Ph.D.)

..... Chair

(Asst. Prof.Amporn Jariyapongskul, Ph.D.)

..... Co-advisor

(Ulisa Pachekrepapol, Ph.D.)

..... Committee

(Srisombat Puttikamonkul, Ph.D.)

..... Co-advisor

(Kittiwut Kasemwong, Ph.D.)

Title	CHARACTERIZATION OF PROBIOTIC LACTIC ACID BACTERIA WITH CHOLESTEROL-LOWERING POTENTIAL *IN VITRO* AND *IN VIVO* AND THEIR APPLICATION IN DAIRY PRODUCT
Author	PREEYARACH WHISETKHAN
Degree	MASTER OF SCIENCE
Academic Year	2018
Thesis Advisor	Assistant Professor Malai Taweechotipatr , Ph.D.

Hyperlipidemia or high cholesterol increases the risk of developing heart disease, coronary artery disease and artherosclerosis, In addition to using drugs and controlling food. Specific strains of probiotic lactic acid bacteria have been studied for beneficial of heath. One of specific probiotic properties is able to reduce serum cholesterol. Seventy-five strains of lactic acid bacteria were isolated from traditional fermented Thai food products of Thailand are screened for bile salt hydrolase activity.Two bacteria strains (F1-1 and F23-5) which exhibit higher bile salt hydrolase activity were identified based on phenotypic and 16s rDNA gene sequence analysis.

The F1-1 strain was identified as *Lactobacillus pentosus* DSM 20314^{T} and F23-3 was *Enterococcus faecium* CGMCC 1.2136^{T}. Characterization and identification of acid and bile tolerance of selected strain had survived and viability at pH 3.0, pH 4.0, bile acid concentration of 0.3%, 0.8%, and did not survive at pH 2.0. F1-1. F23-5 isolates were selected to determine their serum lipid profile in high-fat fed rat. The group of rat fed high-fat with the F23-5 isolate group exhibited total cholesterol level 48.2 mg/dl, the significant lower total cholesterol level in blood sample compare with rat fed high-fat group was 92 mg/dl . Intestinal microbiota analysis of exhibited that pre-treatment and after-treatment were only minor changes of viable bacteria count but similar bacterial type in the intestinal. F23-5 isolate was abile to decrease total cholesterol level in rat fed high fat, therefor it was selected to use fermentation of milk product. This fermentation milk product by F23-5 (*enterococcus faecium*) were

investigated of their quality parameters, the result showed that they exhibited good quality similar to commercial fermented milk product.

Keyword : Probiotic Lactic acid bacteria Cholesterol

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my thesis advisor, Assist. Prof. Malai Taweechotipatr, Ph.D., for this valuable guidance, constant encouragement and always being very understanding me. Her excellent advice, kind guidance, encouragement throughout the period of this study.

My deepest thanks are expressed to my thesis co-advisor, Dr. Ulisa Pachekrepapol, Ph.D, from Srinakharinwirot University, her providing the milk product using throughout my research. And Dr. Kittiwut Kasemwong, Ph.D. from National Nanotechnology Center (Nano tech).

I also would like to thank the department of Microbiology, Faculty of medicine, Srinakharinwirot University for providing equipments and instruments on my research. Researchers and staffs Faculty of Phamaceutical Science, Srinakharinwirot University and Faculty of Phamaceutical Science, chulalongkorn University for their kind suggestions, helpfulness and encouragement.

Finally, I am very grateful to thank my parents and my friends for their love, support and understanding.

PREEYARACH WHISETKHAN

TABLE OF CONTENTS

CONTENT OF TABLES

Page

CONTENT OF FIGURES

Page

CHAPTER I
INTRODUCTION

Probiotics are live microorganisms that provide health benefits to the host when ingested in appropriate amounts.[1] The Food and Agricultural Organization and World Health Organization's (FAO/WHO) defined probiotic bacteria as "live micro-organisms which, when administered in adequate amounts confer a health benefit on the host".[2] Probiotics have multiple and diverse effects on the host, the benefits provided by probiotics are strain-specific, therefore no such probiotic strain can have all proposed benefits, not even strains of the same species.[3] Properties of probiotics include improvement of gastrointestinal micro-flora, modulation of immune system, cancer prevention, treatment of irritable bowel syndrome, alleviation of postmenopausal disorders, antihypertensive effects and reduction of serum cholesterol.[4] One of the most significant groups of probiotic organisms is the lactic acid bacteria (LAB), commonly used in fermented dairy products. These bacteria have a long history of safe use in foods.[5]

Lactic acid bacteria are widespread microorganisms which can be found in any environments rich mainly in carbohydrates. They are the normal microbiota or micro-flora of the gastrointestinal and genitourinary tracts of human and animal.[6, 7] LAB are gram positive, rods or cocci, non-spore forming, acid tolerant, low GC containing, anaerobic or micro-aerophilic bacteria and the modes of glucose fermentation are homo-fermentative and hetero-fermentative.[8] LAB can be found in dairy products, fermented meats, fermented vegetables, and beverages.[9] Many strains of LAB are among important groups of microorganisms used in the foods and feed industries. Some strains of LAB are known as probiotics that exhibit potential health and nutritional benefits. Their ability is proven to be effective against diarrhoea, irritable bowel disorder and allergies. Stimulation of immunity, reduction of lactose intolerance symptom and reduction of cholesterol are also proven.[10] The most commonly used probiotics of lactic

acid bacteria are *Lactobacillus*, *Bifidobacterium*, *Pediococcus*, *Lactococcus* and *Streptococcus*.[11, 12]

Cholesterol in the human body that elevates levels of the blood cholesterol or hypercholesterolemia may lead to atherosclerosis and pose a major risk of developed cardiovascular diseases (CVDs).[13] World Health Organization's (WHO) reported that cardiovascular diseases were responsible for 46% of deaths worldwide, accounting for 7.25 million deaths a year. By the year 2030, cardiovascular diseases will affect approximately 23.3 million people around the world.[14] Serum total cholesterol (TC) levels are correlated with cardiovascular diseases risk factor over a broad range of cholesterol values.[15] Mechanisms for cholesterol reduction by probiotics have been proposed; deconjugation of bile salt by bile salt hydrolase (BSH), assimilation of cholesterol into bacteria cell membranes and production of short-chain fatty acids (SCFAs) by the growth of probiotics.[16]

Bile salt is a yellow-green aqueous solution whose major constituents include bile acids, cholesterol, phospholipids, and the pigment biliverdin.[17] Cholesterol, a precursor of bile acids, is synthesized in hepatocytes of the liver, stored and concentrated in the gallbladder, and released into the duodenum after food intake.[18] Bile salt is further metabolized by the liver via conjugation (N-acyl amidation) to glycine or taurine. Bile functions as a biological surfactant that emulsifies and solubilizes lipids, thereby playing an essential role in fat digestion.[19] Conjugated bile salt are absorbed into the portal bloodstream and are taken up by hepatocytes, reconjugated, and resecreted into bile. Conjugate bile salt is catalyzed by bile salt hydrolase from GI microbiota and probiotics.

Bile salt hydrolase activity is responsible for the deconjugation of bile salt by hydrolysis of the amide bond of the conjugated bile salt, and liberation of the glycine/taurine moiety from the steroid core. Deconjugated bile salts are less soluble and less efficiently reabsorbed by the intestinal lumen than their conjugated counterparts, thus it results in excretion of larger amounts of free bile acids in feces.[20] Therefore, the deconjugation of bile salt lead towards a reduction in serum cholesterol

either by increasing the demand of cholesterol for de novo synthesis of bile salt to replace that lost in feces.[21]

Some probiotic LAB are able to reduce serum cholesterol levels. This effect can be ascribed to an enzymatic deconjugation of bile salt.[22] Based on the enzymatic ability of probiotics to deconjugate bile salt, many experiments have *in vitro* and *in vivo* to investigate cholesterol lowering effect of lactic acid bacteria. Some strains of probiotic LAB were able to deconjugation bile salt by bile salt hydrolase *in vitro*. Previous studies showed that consumption of fermented milk products containing lactobacilli or bifidobacteria has led to a decrease in the concentration of serum cholesterol in humans.[23]

Several species of LAB are potential microorganisms and have been widely applied in food fermentation worldwide. Fermentation is generally considered as a safe and acceptable preservation technology of foods. Fermentation using LAB can be categorized into two groups based on the raw materials used, non-dairy and dairy fermentation. LAB have a role in milk fermentation to produce acid which is important as preservative agents and generating flavor of the products. The main purpose of milk fermentation using LAB is to prolong its shelf-life as well as to preserve the nutritious component of milk.

Specific strains of probiotic lactic acid bacteria have been beneficial of heath and Specific properties of probiotic lactic acid bacteria strains are able to reduce serum cholesterol. In this study, LAB will be isolated from Thai fermented foods. The selected isolates will be screened for potential LAB with bile salt hydrolase activity and then assessed the effects of each strain on rats fed with high-cholesterol diet. The survival of the strains after passage through rat intestines will be determined, and addition of lactic acid bacteria strains in milk will be performed and the quality parameters of fermented milk products will be studied.

Hypothesis

Specific strains of probiotic lactic acid bacteria are able to reduce cholesterol and can be used in probiotic dairy products.

Objective

1. To isolate and select lactic acid bacteria obtained from Thai fermented food.
2. To screen lactic acid bacteria for bile salt hydrolase activity
3. To characterize and identify probiotic lactic acid bacteria strains
4. To study the effects of probiotic lactic acid bacteria strains on rats feeding with high-cholesterol diet.
5. To study the addition of probiotic lactic acid bacteria strains in milk and the quality parameters of probiotic lactic acid bacteria to fermented milk.

CHAPTER II
LITERATURE REVIEW

1. Definition of probiotics

Probiotics are live microorganisms that are good for health, especially digestive system and support body's ability to absorb nutrients and fight infection.[24] The word "probiotic" is a derived of two Greek words: "pro," to signify promotion of and "biotic," which means life.[25] According to the definition by the World Health Organization (WHO), probiotics are ‘‘live microbial food supplements which, when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). One of the most significant groups of probiotic organisms are the lactic acid bacteria (LAB), these bacteria have a long history of safe use in foods.[26] The viability and metabolic activity of these microorganisms must be maintained during all the food processing stages, from manufacture to ingestion, which also can survive in the gastrointestinal tract.[27]

1.1 General properties of probiotics

Probiotics must be safe for their intended use. The 2002 FAO/WHO guidelines recommend that, though bacteria may be generally recognized as safe (GRAS), the safety of the potential probiotic should be assessed by the minimum required tests: [28]

Determination of antibiotic resistance patterns

Assessment of side effects during human studies

Epidemiological surveillance of adverse incidents in consumers (after market)

If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production. One possible scheme for testing toxin production has been recommended by the EU Scientific Committee on Animal Nutrition.[29]

If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required

1.1.1 Resistance to hydrochloric acid, bile

Acid and Bile tolerance is one of the most crucial properties used to select potentially probiotic bacteria strains. As it determines its ability to survive in the small intestine and colonize the host, and consequently its capacity to play its functional role as a probiotic.

1.1.2 Colonize of the gastrointestinal tract

Probiotic bacteria must exhibit a number of functional characteristics, including the resistance to gastric acidity, bile tolerance and the ability to adhere to the intestinal epithelium cell has been considered as one of the selection criteria for probiotic strains. Some of the strains to adhere to Caco-2 cells, for example *L. acidophilus* LA1, *Lactobacillus* GG and *L. rhamnosus* LC-705.

1.1.3 Modulation of normal-flora

Probiotics to successfully exert its benefit on the host's gut microbiota it should be able to remain viable during storage and also be capable of surviving, and potentially colonizing, the host's intestinal environment. probiotics and faecal microbiota transplantation, and modulating the intestinal community in a beneficial way.

1.1.4 Anti-microbial

The antimicrobial or antagonistic activity of probiotics is an important property that includes the production of antimicrobial compounds, competitive exclusion of pathogens, enhancement of the intestinal barrier function.

2. Lactic acid bacteria

Lactic acid bacteria are widespread microorganisms which can be found in any environment rich mainly in carbohydrates such as fermented foods, dairy products and non-dairy products, yogurt, cheese, sauerkraut, sausage and other fermented foods. These bacteria produce lactic acid as the major metabolic end product of carbohydrate fermentation. In human and animal, lactic acid bacteria are symbiotic and are found in the gut flora.[30]

Lactic acid bacteria are gram-positive bacteria, non-spore, aero tolerance, acid-tolerant with a DNA base composition of less than 53% G+C. Their morphology is divided to rod and cocci shape. The mode of glucose fermentation is homo-fermentation and hetero-fermentation. The homo-fermentative lactic acid bacteria convert carbohydrate to lactic acid as the only or major end product. [31]

Lactic acid bacteria are amongst the most important groups of microorganisms used in the food industry. Lactic acid bacteria have been used in food preservation and modification of organoleptic characteristics of foods, such as flavors and texture, with their generally recognized as safe (GRAS) status. Nowadays, lactic acid bacteria have important role in the industry for the synthesis of pharmaceuticals, chemicals, or other products.[32]

2.1 Classification of lactic acid bacteria

The general basis of classification of lactic acid bacteria is connected with the work of Orla-Jensen. This classification system at the genus level first divides the LAB, according to morphology (cocci or rods, tetrad formation), mode of glucose fermentation (homo- or heterofermentation), growth at certain “cardinal” temperatures (e.g., 10ºC and 45ºC), and form of lactic acid produced (D, L, or both).[33] Nowadays, to determine the sequence of long stretch of rRNA (~1500 bases of 16S rRNA) from bacteria comparisons of these sequences are currently the most powerful and accurate technique for determining phylogenetic relationships of microorganisms. In addition, rRNA sequencing is becoming an important aid in the classification of lactic acid bacteria, as exemplified by the descriptions of new genera.[34]

The characteristics of LAB are gram-positive, catalase Negative, anaerobic or microaerophilic, acid-tolerant and non-sporulating rods and cocci. LAB were called 'milk–souring organisms', and often negatively associated with loss of food and feed due to fermentation.[35] LAB are most widely studied and are exploited in numerous industrial applications ranging from starter cultures in the dairy industry to probiotics in dietary supplements and bio-conversion agents.

LAB belong to the order Lactobacillales and include genera: Aerococcus, Alloiococcus, Carnobacterium, Enterococcus,Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Symbiobacterium, Tetragenococcus, Vagococcus and Weissella, which are alllow guanine–cytosine content organisms (31–49 %).[36] LAB in Actinobacteria phylum only includes species of Bifidobacterium genus[37] Characteristics of LAB are summarized in Table 1.

2.2 Metabolism of Lactic Acid Bacteria

Lactic acid bacteria degrade different carbohydrates and related compounds primarily to lactic acid coupled with energy (adenosine-tri-phosphate=ATP) production pathway. Lactic acid bacteria utilize carbohydrates (e.g., glucose) to form lactic acid by either the homo fermentative or hetero fermentative pathway. [38] In addition to the main metabolic pathways of LAB connected with fermentation of carbohydrates, lactic acid bacteria nitrogen metabolism is connected with hydrolysis and synthesis of proteins.[39] Lactic acid bacteria may also produce compounds with antimicrobial activity, and flavor compounds in dairy products and baked goods.

Table 1 Genera of LAB associated with food and their physiological

Table 1 Genera of LAB associated with food and their physiological characteristics

Family	Genera	Characteristics								
		Shape	CO_2 from gucose	Growth at 10 °C	Growth at 45 °C	Growth in 6.5 % NaCl	Growth in 18 % NaCl	Growth at pH 4.4	Growthat pH 9.6	Type of lactic acid
Aerococcaceae	Aerococcus	Cocci (tetrads)	-	+	-	+	-	–	+	L
Carnobacteriaceae	Carnobacterium	Rods	-	+	-	ND	-	ND	-	L
Enterococcaceae	Enterococcus	Cocci	-	+	+	+	-	+	-	L
	Tetrageonococcus	Cocci (tetrads)		+	-	+	+	Variable	+	L
	Vagococcus	Cocci		+	-	-	-	ND	-	ND
	Pediococcus	Cocci (tetrads)	-	Variable	Variable	Variable	-	+	-	D, L, DL
Lactobacillaceae	Lactobacillus	Rods	Variable	Variable	Variable	Variable	-	ND	L	D, L, DL
Leuconostocaecae	Leuconostoc	Cocci	+	+	-	Variable	-	Variable	-	D
	Oenococcus	Cocci	+	+	-	Variable	-	Variable	-	D
	Weissella	Cocci	+	+	-	Variable	-	Variable	-	D, DL
Streptococcaceae	Lactococcusb	Cocci	-	+	-	-	-	Variable	-	L
	Streptococcus	Cocci	-	-	Variable	-	-	-	-	L

2.3 Application of Lactic acid bacteria

Lactic acid bacteria have an important role in the industry for the synthesis of chemicals, pharmaceuticals, or other useful products. LAB have beneficial effects in the food industry, the products of fermented foods are based on the use of starter culture of lactic acid bacteria. They produce antimicrobial substance, sugar polymers, sweeteners, aromatic compounds, vitamins, enzymes, and exhibit probiotic properties.[40] Use of LAB as functional ingredients in the industries is shown in Figure 1. Today in the developed world, lactic acid bacteria are mainly associated with fermented dairy products.

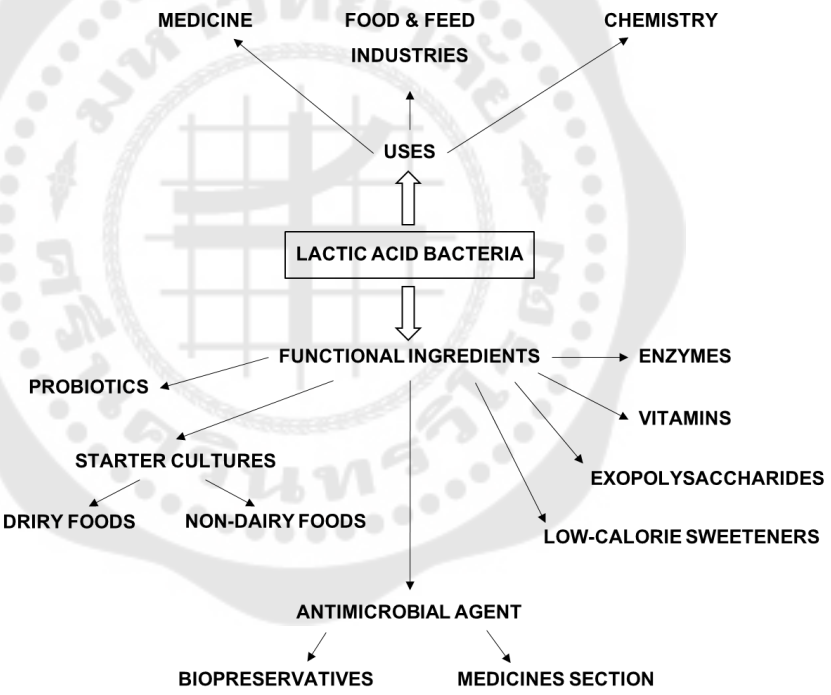


Figure 1 Uses and Functional Ingredients of Lactic Acid Bacteria

Sauce: Florou-Paneri P, Christaki E, Bonos E. (2012). Lactic Acid Bacteria as Source of Functional Ingredients. 589-614.

2.4 Health benefit of Lactic Acid Bacteria

Lactic acid bacteria probiotic strains have the strongest human health efficacy in the management of lactose intolerance, rotaviral diarrhea and antibiotic associated diarrhea. [41] LAB can stimulate the immune system, maintain healthy intestinal microflora, inhibit some pathogen, reduce inflammation,[42] reduce blood cholesterol leading to decreased blood pressure.[43] Health benefits of probiotic LAB are summarized in Table 2.

Table 2 Lactic acid bacteria derived probiotics and human health

Lactic Acid Bacteria	Effects on human health
Lactobacillus acidophilus	Secretes lactic acid which reduces the pH of the gut and inhibits the development of pathogens (*Salmonella* spp, *E.coli*).
Lactobacillus johnsonii	Effective in inhibition of *H. pylori* and against inflammation
Lactobacillus plantarum	Produces short-chain fatty acids that block the generation of carcinogenic agents by reducing enzyme activities
Lactobacillus fermentum	Effective in restoration of a normal microflora. Effective against bacterial vaginosis flora
Lactobacillus reuteri	Reduces the duration of diarrhea
Enterococcus faecium	Can reduce blood cholesterol leadingto decreased blood pressure

2.5 Lactic Acid Bacteria in Dairy Industry

The group of lactic acid bacteria (LAB) occupies a central role in these processes, and has a long and safe history of application and consumption in the production of fermented foods and beverages.[44] In the new generation of fermented foods, LAB with diverse physiological and metabolic traits are combined. Their metabolic and technological properties are often different from those of traditional starter cultures, thus appropriate production processes must be developed.[45]

In dairy industry, various industrial strains of LAB are used as starter cultures. Starter cultures of LAB were obtained from a sequence activities and passed a process of isolation, selection and confirmation. The most important properties of LAB are their ability to acidify milk[46] and to generate flavor and texture, by converting milk protein due to their proteolytic activities.[47, 48] The mild acid taste and pleasant freshness, are characteristics of fermented milk products such as yoghurt and cheese.

LAB have been used in the fermentation of dairy products, as a simple and safe way of preserving such foods. The main species of LAB that can potentially be used as probiotic cultures in dairy products belong to the Lactobacillus spp. (*L. acidophilus, L. lactis, L. casei, L. plantarum, L. rhamnosus, L. reuteri, L. delbrueckii* subsp. *bulgaricus*) or to the Enterococcus spp. [49] A minimum viable LAB count of 10^6 CFU/g in fermented dairy food is recommended for the claimed health benefits. LAB strains used in different food products are shown in Table 3.

Table 3 Fermented foods and beverages and their associated lactic acid bacteria [50]

Type of fermented product	Lactic acid bacteria
Dairy products	
-Hard cheeses without eyes	*L. lactis* subsp. *lactis*, *L. lactis* subsp. cremoris
-Cheeses with small eyes	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *lactis* var. *diacetylactis*, *L. lactis* subsp. *cremoris*, *Leuc. mesenteroides* subsp. *cremoris*
-Swiss- and Italian-type cheeses	*Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus*, *Lb. casei*, *Lb. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*
-Butter and buttermilk	*L. lactis* subsp. *lactis*, *L. lactis* subsp. *lactis* var. *diacetylactis*, *L. lactis* subsp. *cremoris*, *Leuc. mesenteroides* subsp. *cremoris*
-Yoghurt	*Lb. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*
-Fermented, probiotic milk	*Lb. casei*, *Lb. acidophilus*, *Lb. rhamnosus*, *Lb. johnsonii*, *B. lactis*, *B. bifidum*, *B. breve*
-Fermented sausage (Europe)	*Lb. sakei*, *Lb. curvatus*
-Fermented sausage (USA)	*P. acidilactici*, *P. pentosaceus*
Fermented fish products	*Lb. alimentarius*, *C. piscicola*
Fermented vegetables	
-Sauerkraut	*Leuc. mesenteroides*, *Lb. plantarum*, *P. acidilactici*
-Pickles	*Leuc. mesenteroides*, *P. cerevisiae*

Table 3 (continued)

Type of fermented product	Lactic acid bacteria
Fermented vegetables	
-Fermented olives	*Leuc. mesenteroides, Lb. pentosus, Lb. plantarum*
-Fermented vegetables	*P. acidilactici, P. pentosaceus, Lb. plantarum, Lb. fermentum*
Soy sauce	*T. halophilus*
Fermented cereals	
-Sourdough	*Lb. sanfransiscensis, Lb. farciminis, Lb. fermentum, Lb. brevis, Lb. plantarum, Lb. amylovorus, Lb. reuteri, Lb. pontis, Lb. panis, Lb. alimentarius, W. cibaria*
Alcoholic beverages	
-Wine (malolactic fermentation)	*O. oeni*
-Rice wine	*Lb. sakei*

B.=*Bifidobacterium*, C.=*Carnobacterium*, L.=*Lactococcus*, Lb.=*Lactobacillus*, Leuc.=*Leuconostoc*, O.=*Oenococcus*, P.=*Pediococcus*, S.=*Streptococcus*, T.=*Tetragenococcus*, W.=*Weissella*.

3. Lactic acid bacteria as probiotics

Microorganisms as commercial probiotics are mainly of lactic acid bacteria with over one hundred species recognized, for example: *L. acidophilus*, *L. rhamnosus, L.reuteri, L. casei, L. plantarum, L. bulgaricus, L. delbrueckii, L. helveticus.* [50] Probiotics are subject to regulations contained in the general food law, according to which they should be safe for human and animal health.[51] In the USA, microorganisms used for consumption purposes should have the GRAS (Generally Regarded As Safe) status, regulated by the FDA (Food and Drug Administration). In Europe, EFSA introduced the term of QPS (Qualified Presumption of Safety).[52] Probiotics can colonize

the gastrointestinal and exert their beneficial effect in the long term, i.e. probiotics can be used to treat a variety of mucosal surface infections.

4. Properties of probiotics

Functional properties of probiotics have been demonstrated for various therapeutic applications. Many clinical studies have proven the effectiveness of probiotics for treatment of diseases such as obesity, insulin resistance syndrome, type 2 diabetes, and non-alcoholic fatty liver disease. Furthermore, the positive effects of probiotics on human health have been demonstrated by increasing the body's immunity (immunomodulation). Probiotics for treatment of diseases are shown in Table 4. Probiotic lactic acid bacteria derived probiotics have potential health benefits in the following:

1.Diarrheal diseases

A variety of probiotic bacteria, mainly lactobacilli have been used in the treatment and prevention of antibiotic associated diarrhea. Probiotics prevent diarrhea due to their, due to their direct or indirect interaction with the enterotoxins.[53]

2.Inflammatory bowel disease

LAB may affect positively the intestinal mobility and relieve constipation, possibly through a reduction of the intestinal pH, reduce the risk of pouchitis to decrease mucosal inflammation. Among other typical treatments *L. rhamnosus* is used aiming at decreasing the rate and the severity of disease after surgery.[54]

3 Irritable bowel syndrome

Irritable bowel syndrome is described as a heterogeneous group of gastrointestinal symptoms. Probiotics such as *L. plantarum* strain 299V and *E. faecium* PR88 could be used as an effective treatment against this syndrome such as *L. plantarum* strain 299V and *E. faecium* PR88 could be effective treatments against this syndrome.[55]

4 Prevention of colon cancer

Probiotics may be attributable to a combination of mechanisms like the induction of pro- or anti-inflammatory and secretary responses that could inhibit

carcinogenesis. Probiotics can protect against the development of colon cancer in humans.[56] It is hypothesized that the strains tested may have anti-carcinogenic effects by reducing the activity of the enzyme β-glucuronidase.[57]

5. Lactose intolerance

Lactose intolerance is most common disorder of the intestinal carbohydrate digestion. Probiotics can improve the lactose digestion by reducing the intolerance symptoms and slowing corocecal transit.[58]

6. Blood cholesterol

Probiotic bacteria significantly reduce blood cholesterol and increase resistance of low density lipoprotein oxidation, leading to decrease of blood pressure. Strains such as *L. acidophilus, L. plantarum* and *Enterococcus faecium* reduced total-cholesterol and LDL-cholesterol in plasma.[59, 60]

Table 4 probiotics for treatment of diseases.

Subjects	Microorganism	Time of administration	Main Outcome
Obesity			
50 obese adolescents	*L. salivarius* Ls-33	12 weeks	Increase in the ratios of Bacteroides, Prevotellae and Porphyromonas.
50 adolescents with obesity	*L. salivarius* Ls-33	12 weeks	No effect.
87subjects with high BMI	*L.gasseri* SBT2055	12 weeks	Reduction in BMI, waist, abdominal VFA, and hip circumference
210 adults with large VFA	*L.gasseri* SBT2055	12 weeks	Reduction in BMI and arterial BP values

Table 4 (continued)

Subjects	Microorganism	Time of Administration	Main Outcome
75 subjects with high BMI	*L. acidophilus* La5, *B. lactis* Bb12, *L. casei* DN001	8 weeks	Changes in gene expression in PBMCs as well as BMI, fat percentage, and leptin levels.
70overweight and obese subjects	*E. faecium* and 2, *S. thermophilus*	8 weeks	Reduction in body weight, systolic BP, LDL-C.
Insulin resistance syndrome			
28 patients with IRS	*L. casei* Shirota	12 weeks	No effect.
30 patients with IRS	*L. casei* Shirota	12 weeks	Significant reduction in the
24 PM women with IRS	*L. plantarum*	12 weeks	Glucose and homocysteine levels were significantly reduced
Type 2 diabetes			
40 patients with T2D	*L. planatarum* A7	8 weeks	Decreased methylation process, SOD, and 8-OHDG.
45 patients with T2D	*L. acidophilus* La-5,*B. animalis* subsp. *lactis* BB-12	6 weeks	Significant difference mean changes of HbA1c, TC, and LDL-C.

Table 4 (continued)

Subjects	Microorganism	Time of Administration	Main Outcome
44 patients with T2D	*L. acidophilus* La-5, *B. animalis* subsp. *lactis* BB-12	8 weeks	Increased HDL-C levels and decreased LDL-C/HDL-C ratio.
64 patients with T2D	*L. acidophilus* La5,*B. lactis* Bb12	6 weeks	Reduced fasting blood glucose and antioxidant status.
60 patients with T2D	*L. acidophilus* La5,*B. lactis* Bb12	6 weeks	TC and LDL-C Improvement.
45 males with T2D	*L. acidophilus* NCFM	4 weeks	No effect.
Non-alcoholic fatty liver disease			
20obese children with NAFLD	*L. rhamnosus* GG	8 weeks	Decreased ALT and PG-PS Ig Ag antibodies
28 adult individuals with NAFLD	*L. bulgaris*, *S.thermophilus*	12 weeks	Decreased ALT and γ-GTP levels.
72 patients with NAFLD	*L.acidophilus* La5, *B. breve* subsp. *Lactis* Bb12	8 weeks	Reduced serum levels of ALT, ASP, TC, and LDL-C.
44obese children with NAFLD	Bifidobacterium, *Lactobacillus, S. thermophiles*	16 weeks	Improved fatty liver severity, decreased BMI, and increased GLP1/aGLP1.

5. Cholesterol

Cholesterol is a waxy, fat-like substance that occurs naturally in all parts of the body in the blood. Cholesterol is present in tissues and in plasma. The body uses cholesterol to protect nerves, make cell tissues, and produce certain hormones.[61] Cholesterol are synthesis of liver and the body also gets cholesterol directly from the food such as eggs, meats, and dairy products. Lipoproteins carry cholesterol in the blood. The two main types that carry cholesterol to and from cells are called low density lipoproteins (LDL-C) and high density lipoproteins (HDL-C).[62] When there is too much cholesterol in the blood, it builds up in the walls of the arteries (plaque). Plaques can also split open, leading to formation of a blood clot that blocks the flow of blood. Over time, it can increase a risk factor in the genesis of atherosclerosis of vital arteries, coronary, and peripheral vascular disease.[63]

5.1 Cholesterol synthesis

Cholesterol like long-chain fatty acids, synthesis starts with acetyl-CoA in the liver. The biosynthesis of cholesterol into five stages. The schematic diagram of cholesterol biosynthesis is shown in figure 2

Stage 1 synthesis of mevalonate from acetyl-CoA : the three acetate units condense to form a six-carbon intermediate, mevalonate.

Stage 2 formation of isoprenoid units from mevalonate by loss CO2. : involves the conversion of mevalonate into activated isoprene units.

Stage 3 condensation of six isoprenoid units from squalene: the polymerization of six 5-carbon isoprene units form the 30-carbon linear structure of squalene.

Stage 4 cyclization of squalene give rise to the parent steroid, lenosteroid : the cyclization of squalene forms the four rings of the steroid nucleus.

Stage 5 the formation of cholesterol: formation of cholesterol from lanosterol takes place in the membranes of the endoplasmic reticulum and involves changes in the steroid nucleus and side chain. Finally, the double bond of the side chain is reduce producing cholesterol.[64]

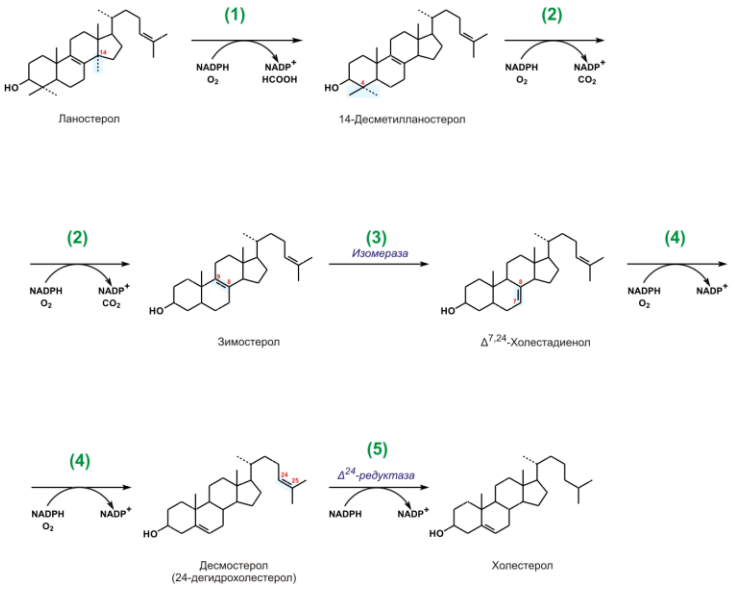


Figure 2 Cholesterol synthesis pathways

Sauce: https://courses.lumenlearning.com/suny-hccc-nutrition/chapter/6-35-cholesterol-synthesis

5.2 Lipoproteins

Lipoproteins are particles that contain triacylglycerol, cholesterol, phospholipids and amphipathic proteins. Lipoproteins can be differentiated on the basis of their density, the degree of lipid in a lipoprotein affects its density—the lower the density of a lipoprotein, the more lipid it contains relative to protein.[65, 66] Four major types of lipoproteins are chylomicrons, very low density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). The fates of lipoproteins produced by the liver is shown in Figure 3

Low-density lipoprotein (LDL)

The function of LDL is to deliver cholesterol to cells, where it is used in membranes, or for the synthesis of steroid hormones. Cells take up cholesterol by receptor-mediated endocytosis. LDL binds to a specific LDL receptor and is internalized

in an endocytic vesicle. Receptors are recycled to the cell surface, while hydrolysis in an endolysosome releases cholesterol for use in the cell. [67]

High-density lipoprotein (HDL).

HDL is involved in reverse cholesterol transport, excess cholesterol is eliminated from the body via the liver by HDL in a process as reverse cholesterol transport which secretes cholesterol in bile or converts it to bile salts. HDL (or really, the HDL precursor) is synthesized and secreted by the liver and small intestine. It gathers cholesterol to form mature HDL, which then returns the cholesterol to the liver via various pathways.[68]

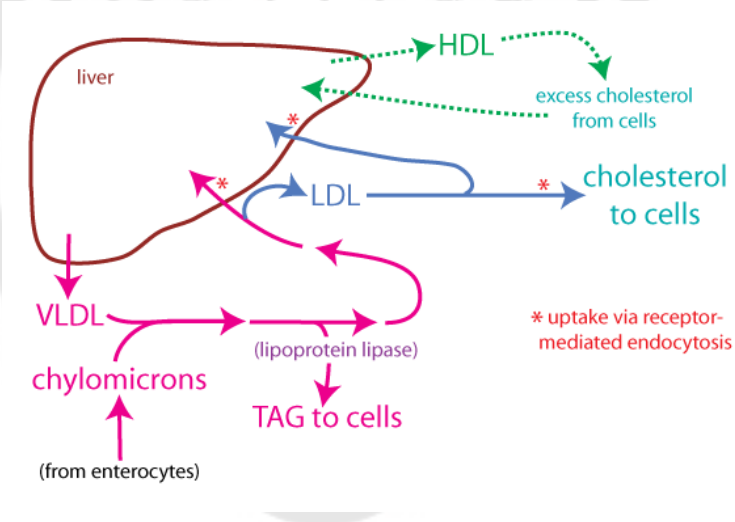


Figure 3 The fates of lipoproteins produced by the liver

Sauce: https://courses.washington.edu/conj/bess/cholesterol/liver.html

5.3 Transport of cholesterol

Cholesterol is transported in the plasma in lipoprotein with the form of cholesteryl esters. Dietary cholesterol is absorbed by the intestine together with dietary unesterified cholesterol and other lipids. Among the cholesterol absorbed, 80-90% are esterified (with long-chain fatty acid) in the intestine mucosa.[69] Dietary cholesterol

equilibrates with plasma cholesterol in day and with tissue in weeks. Cholesterol synthesized and secreted by the liver in VLDLs and these are converted to LDLs through the action of endothelial cell associated lipoprotein lipase. [70, 71] Cholesterol found in plasma membranes can be extracted by HDLs and esterified by the HDL-associated enzyme lecithin-cholesterol acyl transferase, LCAT. The cholesterol acquired from peripheral tissues by HDLs can then be transferred to VLDLs and LDLs via the action of cholesteryl ester transfer protein (CETP) which is associated with HDLs.[72]

5.4 High cholesterol

Cholesterol is an important basic block for body tissues, elevated blood cholesterol is a well-known major hypercholesterolemia contributes to 45% of heart attacks in Western Europe and 35% of heart attacks in Central and Eastern Europe.[73] World Health Organization's (WHO) reported that cardiovascular diseases were responsible for 46% of deaths worldwide, accounting for 7.25 million deaths a year. By the year 2030, cardiovascular diseases will affect approximately 23.3 million people around the world. Serum total cholesterol (TC) levels are correlated with cardiovascular diseases risk factor over a broad range of cholesterol values.[74]

6. Bile salt

Bile is a yellow-green aqueous solution whose major constituents include bile acids, cholesterol, phospholipids, and the pigment biliverdin.[75] Bile salt is synthesized in the liver, stored and concentrated in the gallbladder interdigestively, and released into the duodenum after food intake. Bile salt functions as a biological detergent that emulsifies and solubilizes lipids, principally facilitating the intestine digestion and absorption of dietary fats and fat soluble vitamins.[76]

6.1 Bile salt synthesis

Bile acids are derivatives of cholesterol synthesized in the hepatocyte. Cholesterol, ingested as part of the diet or derived from hepatic synthesis is converted into the bile acids cholic and chenodeoxycholic acids, which are then conjugated to an amino acid (glycine or taurine) to yield the conjugated form that is actively secreted into cannaliculi. [77]

The primary bile salt are synthesized from cholesterol in hepatocytes. The two primary bile acids synthesized in the human liver are cholic acid (CA; 3a, 7a, 12a-trihydroxy-5b-cholan-24-oic acid) and chenodeoxycholic acid (CDCA; 3a,7a-dihydroxy-5b-cholan-24-oic acid). The pathway of bile acid biosynthesis divides early into one subpathway leading to cholyl-CoA, characterized by an extra α-OH group on position 12, and another pathway leading to chenodeoxycholyl-CoA. A second pathway in mitochondria involving the 27-hydroxylation of cholesterol by sterol 27-hydroxylase as the first step is responsible for a significant proportion of the primary bile acids synthesized. The primary bile salt are further metabolized by the liver via conjugation (N-acyl amidation) to glycine or taurine, a modification that decreases the pKa to approximately 5. Thus, at physiological pH, conjugated bile acids are almost fully ionized and may be termed bile salts. [77] Bile salt synthesis is shown in figure 4

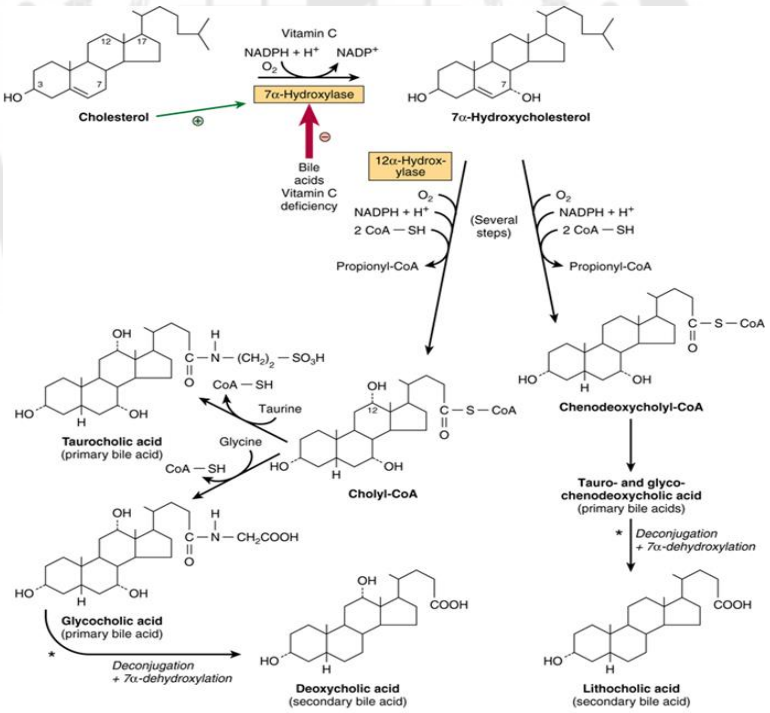


Figure 4 Bile salt synthesis

Sauce: E Merritt M, Donaldson J. (2009) Effect of bile salts on the DNA and membrane integrity of enteric bacteria. 1533 - 41 p.

6.2 Roles of Bile Acids in Fat Digestion and Absorption

Their amphipathic nature enables bile acids to carry out two important functions:

Emulsification of lipid aggregates: Bile acids have detergent action on particles of dietary fat which causes fat globules to break down or be emulsified into minute, microscopic droplets. Emulsification is not digestion, but is of importance because it greatly increases the surface area of fat, making it available for digestion by lipases, which cannot access the inside of lipid droplets.

Solubilization and transport of lipids in an aqueous environment: Bile acids are lipid carriers and are able to solubilize many lipids by forming micelles - aggregates of lipids such as fatty acids, cholesterol and monoglycerides - that remain suspended in water. Bile acids are also critical for transport and absorption of the fat-soluble vitamins.[78]

7. Probiotic bacteria as cholesterol-lowering

Probiotics have been suggested to have cholesterol-lowering effects, especially the strains of genera *Lactobacillus* and *Bifidobacterium.*[22] The mechanisms for cholesterol removal by probiotics have been proposed, such as the cholesterol assimilation into bacterial cell membranes, deconjugation of bile salts by bile salt hydrolase (BSH) and production of short-chain fatty acids (SCFAs) during growth of probiotics.

7.1 Cholesterol-lowering mechanisms of BSH activity

BSH-active probiotic bacteria may act in the intestine as well as in the liver and other organs. BSH-active probiotics have been shown to increase intraluminal bile acid deconjugation, resulting in increased levels of circulating deconjugated bile salts. The increased deconjugation of bile salts decreases cholesterol absorption by enterocytes.[74] BSH activity is responsible for the deconjugation of bile acids, by hydrolysis of the amide bond of the conjugated bile acid, and liberation of the glycine/taurine moiety from the steroid core. Deconjugated bile salts are less soluble and less efficiently reabsorbed by the intestinal lumen than their conjugated

counterparts, thus it results in excretion of larger amounts of free bile acids in feces.[79] Therefore, the deconjugation of bile acids lead towards a reduction in serum cholesterol either by increasing the demand of cholesterol for de novo synthesis of bile acids to replace that lost in feces.[21] BSH activity is specific to the microbiota and is not present in eukaryotic cells, substantiating the importance of the gut microbiota in cholesterol metabolism.

BSH activity can be detected in all *lactobacilli* and *bifidobacteria* strains. BSH is deconjugation of bile acid, which makes the bile salt less soluble and be excreted out as free bile acid via feaces. This will reduce the cholesterol in serum and increase synthesis to replace the lost bile acid.[80] Mechanisms of BSH activity are shown in figure 5, Table 5 summarizes findings for cholesterol lowering effects of probiotics.

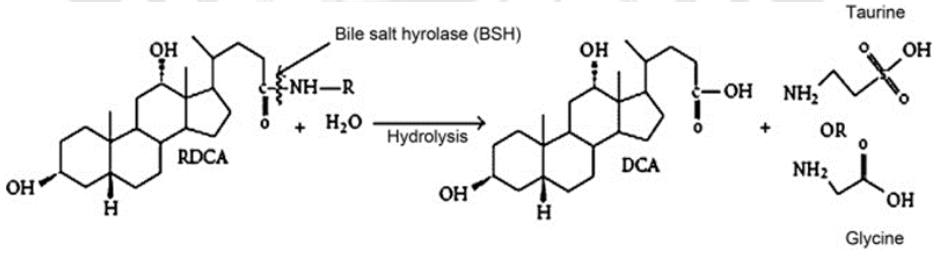


Figure 5 Mechanisms of BSH activity

Sauce: Bhange P, Sridevi N, Bhange DS, Prabhune A, Ramaswamy V. (2014) Immobilization of bile salt hydrolase enzyme on mesoporous SBA-15 for co-precipitation of cholesterol. International Journal of Biological Macromolecules. 63:218-24.

Table 5 summarizes findings for cholesterol lowering effects of probiotics [81]

	Probiotic organism	Experimental system	Major findings
n vivo	Yogurt (unknown)	Human	Reduced total cholesterol and LDL
	Fortified buffalo milk-yogurts with *B. longum*	Rat	Reduced total cholesterol, LDL-cholesterol, and triglyceride
	L. plantarum	Mice	Reduced blood cholesterol Decreased triglycerides
	L. plantarum	Rat	Decreased total cholesterol
	L. plantarum	Rat	Decreased LDL, VLDL, and increased HDL with decrease in deposition of cholesterol and triglyceride in liver and aorta
In vitro	*L. fermentum*	Culture media	BSH activity
	L. plantarum	Culture media	Cholesterol assimilation
	L. acidophilus	Culture media	Assimilation of cholesterol
	L. bulgaricus		Attachment of cholesterol onto cell surface
	L. casei		Disrupt the formation of cholesterol micelle Deconjugation of bile salt Exhibited bile salt hydrolase activity
	L. reuteri	Culture media	Cholesterol assimilation
	L. fermentum		

CHAPTER III
MATERIALS AND METHOD

Materials

1. De Man Rogosa Shape (MRS) media (Oxoid, Basingstoke, Hampshire, UK)
2. Calcium carbonate (CaCo3)
3. Glycerine (Sigma, USA)
4. Skim milk (Difco, USA)
5. Anaerobic gas package (MGC, japan)
6. Anaerobic jar (Mitsubishi, Japan)
7. Light microscope (Nikon, Japan)
8. pH meter (Thermo Scientific, USA)
9. Incubator (Selecta, Spain)
10. Autoclave (Selecta, Spain)
11. Larminar flow hood (Nuaire, USA)
12. Centrifuge (Sartorius Stedim, Germany)
13. Spectrophotometer UV (Shimadzu, Japan)
14. Sodium salt of taurodeoxycholic acid (TDCA) (Sigma, USA)
15. Cacium chloride ($CaCl_2$) (Merck, Germany)
16. Carbohydrates (Merck, Germany)
17. L- arabinose, cellobiose, D-galactose, gluconate, melibiose, α-methy-D-glucoside, Rhamnose,salicin, trehalose, sucose, ribosemaltose
18. Yeast extract (Difco, USA)
19. Peptone (Difco, USA)
20. Beef extract (Difco, USA)
21. Tween 80 (Difco, USA)
22. Agar (Difco, USA)
23. Nitrate (Sigma, USA)
24. L(+) arginine HCl (Sigma, USA)

25. Hydrochloric acid (HCL) (Merck, Germany)
26. Potassium phosphate dibasic (K_2HPO_4) (Merck, Germany)
27. PCR Authorized thermal Cycler (Eppendrof, Germany)
28. PCR DNA fragment extraction kit (Geneaid Biotech, Taiwan)
29. Gel electrophoresis chamber MiniRun GE-100 (HandzhouBioer technology, China)
30. Ox gall (sigma, USA)
31. Eosin methylene blue (EMB) (Difco, USA)
32. Selective Media for Bifidobacteria (BSM) (Difco, USA)

Method

1. Isolation and selection of lactic acid bacteria

Thirty traditional fermented food samples were collected from Bangkok, Thailand. One g of fresh samples were activated and cultivated in 10 ml DeMan Rogosa Shape (MRS) broth and incubated at 37°C for 48 h under anaerobic condition using anaerobic jar. One loop full of broth culture were transferred and streaked on MRS agar plate containing 0.3% calcium carbonate ($CaCO_3$). Single pure colony of lactic acid bacteria were selected by the presence of clear zone around the colony and were be picked up for purification based on different colony morphology. The isolates were firstly screened for catalase activity and Gram staining, and only those that are catalase-negative and Gram-positive were selected. Pure culture were selected and maintained in MRS broth containing 20% glycerol for storage at -80°C and were lyophilized for further studies. [82-84]

2. Screening of lactic acid bacteria for bile salt hydrolase activity

The lactic acid bacteria were screened for bile salt hydrolase (BSH) activity by direct plate assay. Ten µl (10^9 CFU/ml) of a culture grown in MRS broth were spotted onto MRS agar plates supplemented with 0.5% sodium salt of taurodeoxycholic acid (TDCA, Sigma, USA) and 0.37 g/l of calcium chloride ($CaCl_2$)and incubated at 37°C for 48 h under anaerobic condition using anaerobic jar. MRS agar plate without supplementation of TDCA and CaCl2 were used as control. The BSH activity were determined by the diameter of the precipitation zones. The assay were performed in duplicate. [84-86]

3. Characterization and identification of probiotics lactic acid bacteria

3.1 Acid and bile tolerance test

3.1.1 Acid tolerance

Selected lactic acid bacteria isolates were cultivated into MRS broth at 37°C for 48 h under anaerobic condition. Each strain of 10^9 CFU/ml were inoculated into MRS broth at various pH values 2.0, 3.0 and 4.0 adjusting with hydrochloric acid

(1N HCL). The cultures were incubated at 37°c for 3 h under anaerobic condition using anaerobic jar. After incubation, 10-fold serial dilution were performed with addition of phosphate buffer (pH 7.2). One hundred µl of each serial dilution (10^{-4}-10^{-6}) were transferred onto MRS agar plate. Spread plate technique were used and incubated at 37°C for 48 h under anaerobic condition using anaerobic jar. Total viable counts were determined after 3 h incubation and displayed by the log 10 of colonies grown on MRS agar. Unadjusted pH MRS broth (pH 6.5) were used as control. All experiments were done in duplicate and two experiments.[87]

3.1.2 Bile tolerance

Selected lactic acid bacteria isolates were cultivated into MRS broth at 37°C for 48 h under anaerobic condition. Each strain containing 10^9 CFU/ml were inoculated into MRS broth at various concentrations of bile salt (0.3 and 0.8%) using Oxgall. The cultures were incubated at 37°C for 3 h under anaerobic condition using anaerobic jar. After incubation, 10-fold serial dilution were performed with addition of phosphate buffer (pH 7.2). One hundred µl of each serial dilution (10^{-4}-10^{-6}) were transferred onto MRS agar plate. Spread plate technique were used and incubated at 37°C for 48 h under anaerobic condition using anaerobic jar. Total viable counts were determined after 3 h incubation and displayed by the log 10 of colonies grown on MRS agar. Unadjusted pH MRS broth (pH 6.5) were used as control. All experiments were done in duplicate and two experiments.[87]

3.2 Phenotypic characteristics [88]

3.2.1 Acid production (sugar utilization)

Lactic acid bacteria isolates were tested for acid production ability from different carbohydrate sources (L- arabinose, cellobiose, D-galactose, gluconate, melibiose, α-methy-D-glucoside, rhamnose, salicin, trehalose, sucose, ribose, maltose). The basal media containing 0.5% carbohydrates, 0.5% yeast extract, 0.5% peptone, 0.5% beef extract, 0.025% tween 80, 0.5% salt solution and bromocresol purple as an indicator were used. The pH level was adjusted to 6.8. Isolates were cultivated into MRS broth at 37°C for 48 h under anaerobic condition. Each strain of 10^9 cells/ml were inoculated into medium. The culture was incubated at 37°C for 24-48 h under anaerobic condition using anaerobic jar. The basal medium was used as control.

3.2.2 Nitrate reduction

Each strain of selected lactic acid bacteria (10^9 CFU/ml) were inoculated into nitrate medium containing 0.1% KNO_3, 0.3% yeast extract, 0.5% peptone, 0.5% NaCl, 0.01% tween 80 and 0.1% agar. The culture was incubated at 37°C for 24-48h under anaerobic condition using anaerobic jar. The nitrate mediums were used as control.

3.2.3 Arginine hydrolysis

Each strain of selected lactic acid bacteria at the concentration of 10^9cells/ml were inoculated into arginine agar containing 0.1% peptone, 0.3% yeast extract, 0.5% NaCl, 0.03% K_2HPO_4, 1.0% L(+) arginine HCl, 0.001% phenol red, 0.01% tween 80 and 0.1% agar at pH 7.2. The culture was incubated at 37°C for 24-48 h under anaerobic condition using anaerobic jar. The arginine medium was used as control.

3.2.4 Growth at different temperatures

Each strain of selected lactic acid bacteria at the concentration of 10^9 CFU/ml were inoculated into MRS broth, and incubated at 20, 30, 37, 40, 43 and 65°C for 24-48 h under anaerobic condition using anaerobic jar. The MRS broth was used as control.

3.2.5 Growth at pH 2.0, 3.0, 3.5, 4.0, 4.5

Each strain of selected lactic acid bacteria at the concentration of 10^9 cells/ml were inoculated into MRS broth at various pH levels (2.0, 3.0, 3.5, 4.0, 4.5) adjusted with hydrochloric acid. The culture were incubated at 37°C for 24-48 h under anaerobic condition using anaerobic jar.

3.2.6 Growth at salt

Each strain of selected lactic acid bacteria at the concentration of 10^9 CFU/ml were inoculated into MRS broth at various NaCl concentrations, 3.0 and 6.5%. The culture was incubated at 37°C for 24-48 h under anaerobic condition using anaerobic jar.

3.2.7 Gas production

Each strain of selected lactic acid bacteria at the concentration of 10^9 CFU/ml were inoculated into MRS broth with Durham tubes. The culture was incubated at 37°C for 48 h under anaerobic condition by using anaerobic jar. The MRS broth was used as control.

3.2.8 Slime production

Each strain of selected lactic acid bacteria at the concentration of 10^9 CFU/ml were streaked onto slime production medium containing 2% sucrose, 0.5% yeast extract, 0.5% peptone, 2% agar with pH adjusted to 6.8-7.0. The culture was

incubated at 37°C for 24-48 h under anaerobic condition by using anaerobic jar. The slime production medium was used as control.

3.2.9 Starch hydrolysis

Each strain of selected lactic acid bacteria at the concentration of 10^9 CFU/ml were streaked on starch agar containing 2% starch, 0.5% yeast extract, 0.5% peptone, 2% agar and adjust to pH 6.8-7.0. The culture was incubated at 37°C for 24-48 h under anaerobic condition using anaerobic jar. The starch medium was used as control.

3.2.10 Growth rate

Lactic acid bacteria isolates were cultivated onto MRS agar at 37°C for 24 h under anaerobic condition. One loop full of cultured were inoculated into MRS broth (25 ml) and incubated at 37°C for 24 h under anaerobic condition. After that, the inoculum were transferred into 25 ml production media (MRS broth) with a concentration of OD600 = 0.2. The production media was incubated at 37°C for 36 h under anaerobic condition using anaerobic jar. Measurement at OD600 was done every 3 h. All experiment was done in triplicates.

3.3 Genotypic characteristics by 16S rDNA sequencing

Lactic acid bacteria grown at 37°C for 24 h on MRS agar were used for 16S rDNA gene sequences. The 16S rDNA sequences coding region were amplified by PCR in a PCR thermal cycler. The sequences of the PCR products using the prokaryotic 16S ribosomal DNA universal primers 27F(5 ' AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were purified using a Geneaid Gel/PCR DNA fragment Extract Kit (Geneaid Biotech, Bade City, Taiwan). The sequenced analysis of PCR products by Macrogen, Korea were done using universal primers. The similarity of 16S rDNA gene sequences were determined using BLAST software compared to

EzTaxon-e database. Multiple alignments of sequence were performed by CLUSTAL X in BioEdit Sequence Alignment Editor Software. The phylogenetic tree were constructed with neighbor-joining in the MEGA 6 software.

4. Animal study

Thirty male Sprague-Dawley rats aged 6 weeks and weighed 214 - 230 g were used in this experiment. The rats were housed individually in a metal cage under a controlled environment with 23±2°C temperature, 55±5% humidity and maintained on a 12 h light-dark cycle. The rats were fed a commercial diet (082G) containing 27.2% protein, 6.49% fat, 2.17% fiber, 6.63% Ash, 0.479% NaCl, 1.31% Ca and 0.836% P for 1 week. After adaptation period, the rats were randomly selected and assigned to one control group and five experimental groups with five rats each.[80, 89, 90]

(1) Normal group: the rats were fed commercial diet and normal saline.

(2) High-fat diet group: the rats were fed high-fat diet containing 1 ml of lard with egg yolk.

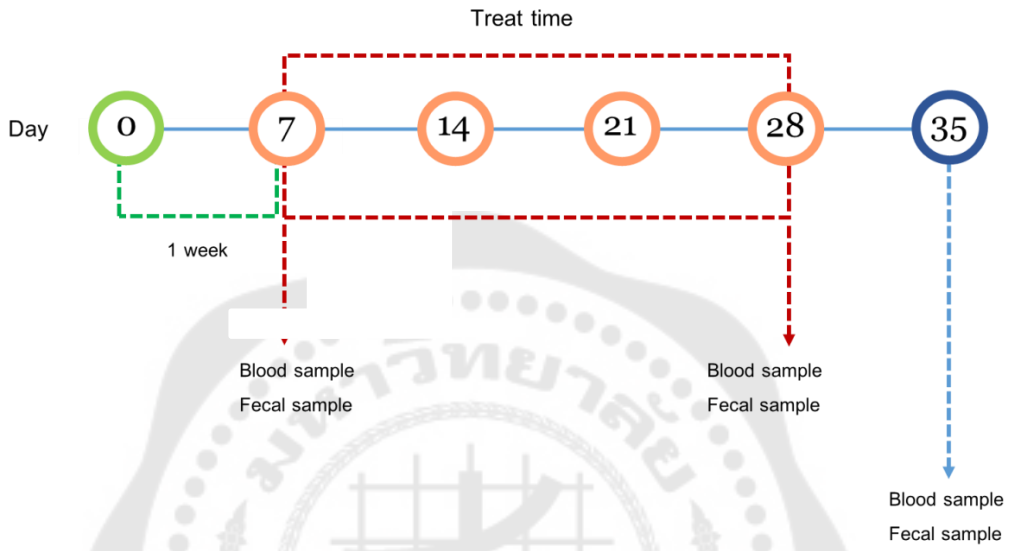
(3) High-fat diet group + probiotic LAB stain F1-1: the rats were fed high-cholesterol diet and 1 ml (10^9 CFU/ml) of probiotic LAB F1-1

(4) High-fat diet group + probiotic LAB stain F23-5: the rats were fed high-cholesterol diet and 1 ml (10^9 CFU/ml) of probiotic LAB F23-5.

(5) Probiotic LAB stain F1-1: the rats were fed commercial diet and 1 ml (10^9 CFU/ml) of probiotic LAB F1-1

(6) Probiotic LAB stain F23-5: the rats were fed commercial diet and 1 ml (10^9 CFU/ml) of probiotic LAB F23-5

The rats were having free access to water and their group specific diet. Body weights were recorded weekly and food intake were recorded daily.



4.1 Assay for serum lipid profile

For analysis of blood lipids, blood samples were collected on days 28, 1 milliliters of each blood sample collected from the heart were transferred to nonheparinized tubes. After storage at 0°C for 30 min, sample tubes were centrifuged (3,000xg) at 4°C for 1 min. The serum were examined the concentrations of serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) by Professional laboratory,Thailand (PROLAB).

4.2 Intestinal microbiota analysis

Fecal sample were collected on days 0, 28. One gram of each sample will be homogenized with 9 ml of PBS (pH 7.2) by vortexing for 10 min, and 10-fold serial dilutions were performed with PBS. Each serial dilution (0.1 of 10^{-4}, 10^{-5}, 10^{-6}) were transferred to the Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and *Bifidobacterium* Selective Media (BSM) for separation of *Escherichia coli*, *Lactobacillus* and *Bifidobacterium*, respectively. Spread plate technique were used and incubated at

37°C for 48 h under anaerobic condition using anaerobic jar. The numbers of colonies were recorded after anaerobic condition. [91, 92]

5. The quality parameters of probiotic lactic acid bacteria in fermented milk

5.1 Fermented milk products using probiotics

Each strain of selected probiotic lactic acid bacteria at the concentration of 10^9 CFU/ml were used for inoculation. The milk supplemented with 10% milk powder and 1% sugar were heated at 85°C for 30 min and allowed to cool to 37°C. The selected strain was added to milk and incubated at 37°C until pH of milk reaches 4.6. Changes of pH during fermentation were measured every 5 min using a digital pH-meter. Quality parameters of fermented milk during storage at 4 ± 1°C were evaluated every 7 days for 28 days. The parameters include the viability, pH, viscosity, texture, syneresis, rheological properties and microstructure.

5.2 Measurement of viability

After incubation, 10-fold serial dilutions were performed with addition of phosphate buffer (pH 7.2). 100 µl of each serial dilution (10^{-4}-10^{-12}) were transferred onto MRS agar plate. Spread plate technique were used and incubated at 37°C for 48 h under anaerobic condition using anaerobic jar. Total viability were counted and displayed as cell numbers by the log 10 of colonies grown on MRS agar.

5.3 Measurement of pH

The pH values of the fermented milk products were determined using a pH meter after calibration with fresh pH 4.0, 7.0 and 10.0 standard buffers.

5.4 Apparent viscosity

Viscosity of fermented milk products were carried out under room temperature (22 ± 2°C) using a Brookfield Viscometer with adjusted rotational speed of 0.5 rpm. The viscosity were read at the 15th second during the measurement.[93]

5.5 Measurement of texture

After inoculation, the samples of fermented milk products were poured into plastic containers of 52 mm in diameter until a height of 50 mm were reached. Texture of fermented milk was determined using a compression test carried out with a TA.XTplus

Texture Analyser (Stable Micro Systems, Surrey, UK). A 20 mm acrylic cylinder probe was used. The test speed is fixed at 1 mm/s and the penetration depth is 10 mm. Firmness were expressed as gram (g), which is a peak force of compression. The texture analysis experiments were repeated three times on different dates.[94]

5.6 Measurement of syneresis

Fermented milk (about 30 g) prepared in centrifuge tubes were centrifuged at 680 x g for 10 min at 4°C. Percent (%) syneresis is calculated as shown in equation.[95]

% Syneresis = Weight of whey (g)/total weight of milk (g) ×100

5.7 Measurement of rheological properties

Dynamic oscillatory measurements were performed with a HAAKE RheoStress1Rheometer (Thermo Sciencetific, Waltham, MA, USA) using a plate and cone geometry with 1 mm gap at 4°C. Amplitude sweeps were carried out with strain ranging from 0.05 to 100% and frequency of 1 Hz. Storage modulus (G') and tan delta (tanδ) were obtained.[96]

6. Statistical analysis

The statistical significance or differences were evaluated by Graph Pad Prism version 8.00 Student's t-test with one-tailed distribution. A p-value ≤ 0.05 was considered statistically significantly. For animal study was used one way ANOVA with comparing each group with control group expressed as mean± SD.

CHAPTER IV

RESULTS

4.1 Isolation and selection of lactic acid bacteria

Twenty four of traditional fermented food samples included Sour meat, Sour pork, Rice fermented, Pickle wild spider flower, Pickle garlic chives, Pickle scallion, Pickle lettuce, kimci, pickled garlic, sliced preserved bamboo shoot, pickled chinese mustard, fermented fish, Pickled ginger, pickled Jujube, Pickled tamarind and Isaan sausage were collected from Bangkok , Roi Et and Chiang Mai Thailand. A total 75 lactic acid bacteria were isolated from traditional fermented Thai foods. These lactic acid bacteria isolates were identified by lactic acid production, gram positive and catalase test. Which all isolate exhibit gram-positive, produce lactic acid and catalase-negative show as on Table 6.

Table 6 Isolation of lactic acid bacteria

Sample	Locations	Isolate	Acid production	Gram reaction	Catalase test
Sour meat (Nham nae)	Bangkok	F1-1	+	Ve+	-
		F1-2	+	Ve+	-
		F1-3	+	Ve+	-
		F1-4	+	Ve+	-
Sour pork (Nham moo)	Bangkok	F2-1	+	Ve+	-
		F2-2	+	Ve+	-
		F2-3	+	Ve+	-
Rice fermented (Kaomark)	Bangkok	F3-1	+	Ve+	-
		F3-2	+	Ve+	-
Picklewild spider flower	Bangkok	F4-1	+	Ve+	-
		F4-2	+	Ve+	-
		F4-3	+	Ve+	-
		F4-4	+	Ve+	-

Table 6 (continued)

Sample	Locations	Isolate	Acid production	Gram reaction	Catalase test
Pickle garlic chives	Bangkok	F5-1	+	Ve+	-
		F5-2	+	Ve+	-
		F5-3	+	Ve+	-
Sour pork (Nham moo)	Roi Et,	F6-2	+	Ve+	-
Sour meat (Nham nae)	Roi Et,	F7-1	+	Ve+	-
		F7-2	+	Ve+	-
		F7-3	+	Ve+	-
Pickle wild spider flower	Roi Et,	F8-1	+	Ve+	-
Pickle scallion	Roi Et,	F9-1	+	Ve+	-
		F9-2	+	Ve+	-
		F9-3	+	Ve+	-
Pickle lettuce	Roi Et,	F10-1	+	Ve+	-
		F10-2	+	Ve+	-
Sour meat (Nham nae)	Roi Et,	F11-2	+	Ve+	-
Sour meat (Nham nae)	Roi Et,	F12-2	+	Ve+	-
		F12-3	+	Ve+	-

Table 6 (continued)

Sample	Locations	Isolate	Acid production	Gram reaction	Catalase test
Pickle garlic chives	Roi Et,	F13-1	+	Ve+	-
Kimci	Bangkok	F14-1	+	Ve+	-
Pickled garlic	Bangkok	F20-1	+	e+	-
		F20-2	+	Ve+	-
		F20-4	+	Ve+	-
		F20-5	+	Ve+	-
Pickled garlic	Bangkok	F20-11	+	Ve+	-
		F20-13	+	Ve+	-
		F20-15	+	Ve+	-
		F20-18	+	Ve+	-
Sliced preserved bamboo shoot	Bangkok	F21-1	+	Ve+	-
		F21-3	+	Ve+	-
		F21-5	+	Ve+	-
		F21-6	+	Ve+	-
		F21-15	+	Ve+	-
Pickled chinese mustard	Bangkok	F22-1	+	Ve+	-
		F22-2	+	Ve+	-
		F22-3	+	Ve+	-
Sour pork	Bangkok	F23-1	+	Ve+	-
		F23-2	+	Ve+	-
		F23-3	+	Ve+	-
		F23-4	+	Ve+	-
		F23-5	+	Ve+	-
		F23-6	+	Ve+	-

Table 6 (continued)

Sample	Locations	Isolate	Acid production	Gram reaction	Catalase test
Pickled fish	Bangkok	F24-1	+	Ve+	-
		F24-2	+	Ve+	-
		F24-3	+	Ve+	-
Pickled ginger	Bangkok	F25-1	+	Ve+	-
		F25-2	+	Ve+	-
		F25-3	+	Ve+	-
Sour pork	Chiang Mai	F26-1	+	Ve+	-
		F26-2	+	Ve+	-
		F26-3	+	Ve+	-
		F26-4	+	Ve+	-
		F26-5	+	Ve+	-
Pickled Jujube	Bangkok	F27-1	+	Ve+	-
		F27-2	+	Ve+	-
Pickled tamarind	Chiang Mai	F28-1	+	Ve+	-
		F28-2	+	Ve+	-
		F28-4	+	Ve+	-
		F28-5	+	Ve+	-
		F28-7	+	Ve+	-
		F28-10	+	Ve+	-
Isaan sausage	Bangkok	F29-1	+	Ve+	-
		F29-2	+	Ve+	-
		F29-3	+	Ve+	-

4.2 Screening of lactic acid bacteria for bile salt hydrolase activity

Out of 75 lactic acid bacterial isolates, were screened for bile salt hydrolase (BSH) activity by direct plate assay. Only 2 isolate of F1-1 and F23-5 were exhibited bile salt hydrolase activity (Table 7). These isolate exhibited high bile salt hydrolase activity by provide precipitation around colony on plate. The presence of bile salt hydrolase (BSH) in probiotics, which also helps to reduce the blood cholesterol level of the host. The result below showed precipitation around colony which diffused into medium plate (Figure 6-7)

Table 7 Lactic acid bacterial strains for bile salt hydrolase activity

No	Sample	Isolate		BSH
1	Sour meat	F1	F1-1, F1-2, F1-3, F1-4	F1-1
2	Sour pork	F2	F2-1, F2-2,F2-3	
3	Rice fermented	F3	F3-1, F3-2	
4	Pickle wild spider flower	F4	F4-1, F4-2, F4-3, F4-4	
5	Pickle garlic chives	F5	F5-1, F5-2, F5-3	
6	Sour pork	F6	F6-2	
7	Sour meat	F7	F7-1, F7-2, F7-3	
8	Pickle wild spider flower	F8	F8-1	
9	Pickle scallion	F9	F9-1, F9-2, F9-3	
10	Pickle lettuce	F10	F10-1, F10-2	
11	Sour meat	F11	F11-2	
12	Sour meat	F12	F12-2, F12-3	
13	Pickle garlic chives	F13	F13-1	
14	kimci	F14	F14-1	
15	pickled garlic	F20	F20-1, F20-2, F20-4, F20-5, F20-11, F20-13, F20-15, F20-18	

Table 7 (continued)

NO	Sample	Isolate		BSH
16	sliced preserved bamboo shoot	F21	F21-1, F21-3, F21-5, F21-6, F21-15	
17	pickled chinese mustard	F22	F22-1, F22-2, F22-3,	
18	sour pork	F23	F23-1, F23-2, F23-3, F23-4, F23-5, F23-6	F23-5
19	pickled fish	F24	F24-1, F24-2, F24-3	
20	Pickled ginger	F25	F25-1, F25-2, F25-3	
21	sour pork	F26	F26-1, F26-2, F26-3, F26-4, F26-5	
22	pickled Jujube	F27	F27-1, F27-2	
23	Pickled tamarind	F28	F28-1, F28-2, F28-4, F28-5, F28-7, F28-10	
24	Isaan sausage	F29	F29-1, F29-2, F29-3	

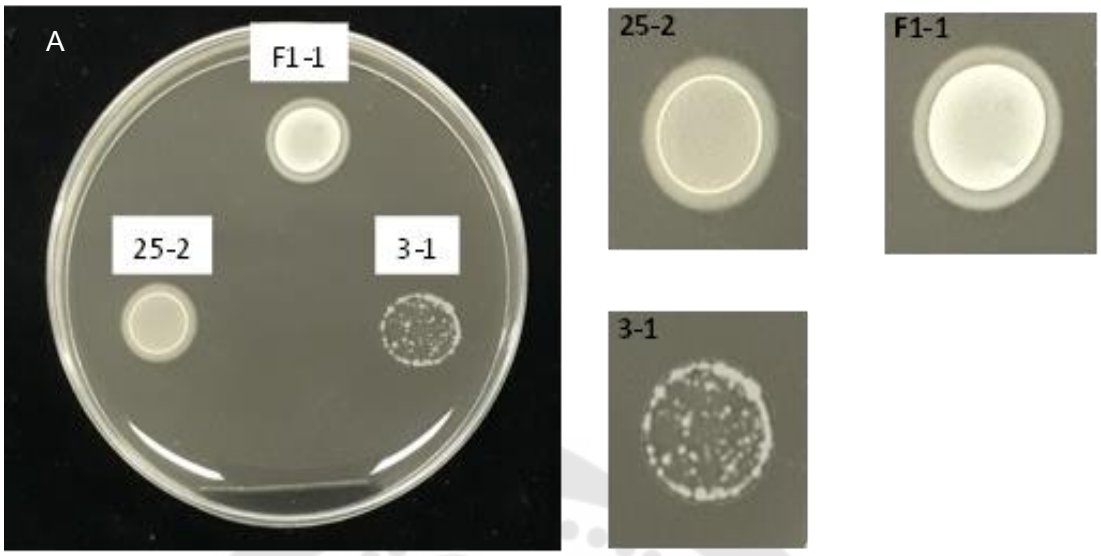


Figure 6 Bile salt hydrolase activity of F1-1 isolate

Precipitation zone around colony on MRS agar plates supplemented with 0.5% sodium salt of taurodeoxycholic acid.

A: bile salt hydrolase activity of lactic acid bacteria

25-2: 25-2 isolate of positive control (*Lactobacillus* sp.)

F1-1: F1-1isolate

3-1: 3-1 isolate of negative control (*Lactobacillus* sp.)

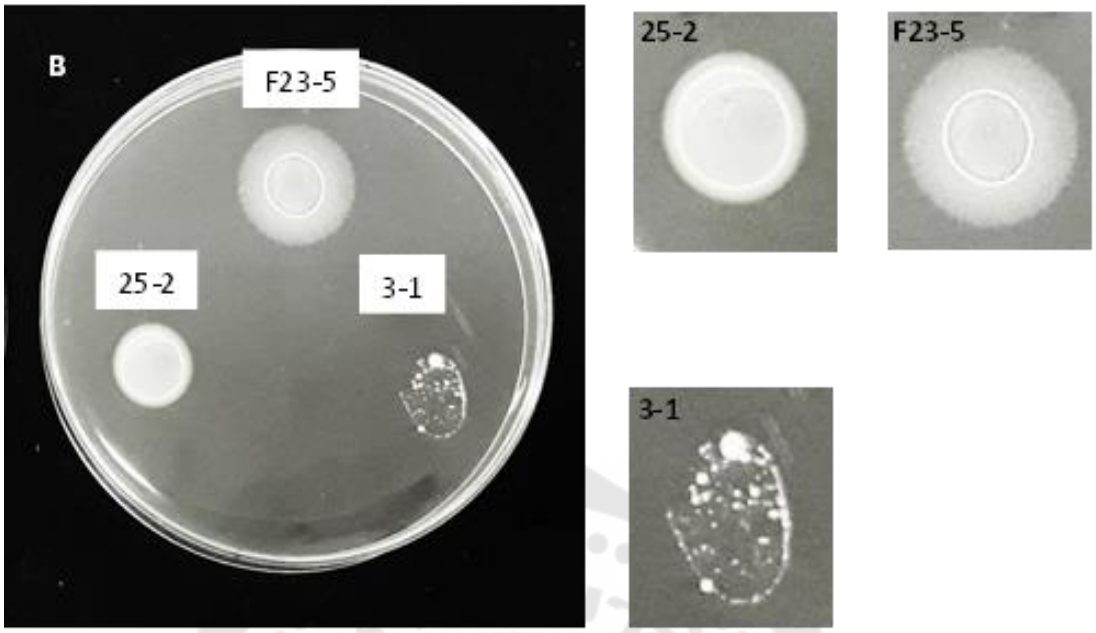


Figure 7 Bile salt hydrolase activity of F23-5 isolate

Precipitation zone around colony on MRS agar plates supplemented with 0.5% sodium salt of taurodeoxycholic acid.

B: bile salt hydrolase activity of lactic acid bacteria

25-2: 25-2 isolate of positive control (*Lactobacillus* sp.)

F23-5: F23-5isolate

3-1: 3-1 isolate of negative control(*Lactobacillus* sp.)

4.3 Characterization and identification of probiotic lactic acid bacteria

4.3.1 Acid and bile tolerance test

Acid and bile tolerance is one of the most crucial properties used to select potentially probiotic bacterial strains. As it determines its ability to survive in the small intestine and colonize the host, and consequently its capacity to play its functional role as a probiotics. Isolation of F1-1 and F23-5 showed the highest bile salt hydrolase activity were selected foe determination of acid and bile tolerance test.

Acid tolerance

As tolerance to acid condition in the stomach affected of potentiality survival probiotic lactic acid bacteria were determined. Lactic acid bacterial isolate which exhibited strongest bile salt hydrolase activity; isolate of F1-1 and F23-5 of lactic acid bacteria were inoculated into MRS broth at various pH values 2.0, 3.0 and 4.0 at 37°C for 3h. After incubated at 37°C for 3 h., all isolates did not survive at pH 2.0 as compared to MRS control at pH 6.5. F1-1 isolate exhibited ability survives at pH 3.0 and pH 4.0 of $4.15x10^{8}$ CFU/ml and $7.67x10^{8}$ CFU/ml respectively. F23-5 isolate exhibited ability to survive at pH 3.0 and pH 4.0 were $4.15x10^{7}$ and $7.67x10^{7}$ CFU/ml respectively.

Bile tolerance

Probiotic bacteria must exhibit a number of functional characteristics, including the resistance to gastric acidity, bile tolerance and the ability to adhere to the intestinal epithelium cell has been considered as one of the selection criteria for probiotic strains. Lactic acid bacterial isolates which exhibited strongest bile salt hydrolase activity, isolate of F1-1 and F23-5 of lactic acid bacteria were inoculated into MRS broth at various concentrations of bile salt 0.3% and 0.8% using Oxgall incubated at 37°C for 3h. After incubated, the survival of F1-1 %bile at 0.3 % bile and 0.8 % bile were $5.46x10^{7}$ CFU/ml and $4.45x10^{7}$ CFU/ml respectively. F23-5 in percentage of %bile at 0.3 % bile and 0.8 % bile were $6.5x10^{7}$CFU/ml and $5.36x10^{7}$CFU/ml respectively.

Table 8 The survival of F1-1 isolate in different in pH values at 37°C for 3 h.

pH value	CFU/ml	SD
MRS o h.	$3.05x10^{8}$	$1.20x10^{8}$
MRS 3 h. (control) pH 6.5	$5.90x10^{8}$	$4.24x10^{8}$
pH 2.0	0	0
pH 3.0	$4.15x10^{7}$	$5.70x10^{7}$
pH 4.0	$7.67x10^{7}$	$1.51x10^{7}$

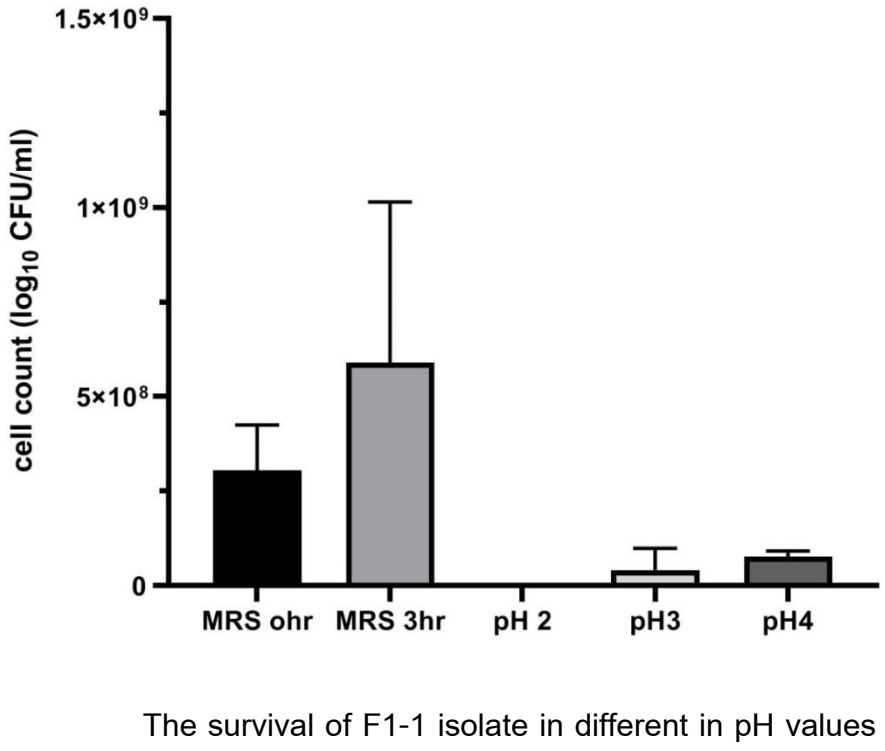


Figure 8 The survival of F1-1 isolate in different in pH values at 37°C for 3 h.

Statistical analysis used one-way ANOVA

Table 9 The survival of F23-5 isolate in different in pH values at 37°C for 3 h.

pH value	CFU/ml	SD
MRS o h.	$6.7x10^8$	$9.90x10^7$
MRS 3 h. (control) pH 6.5	$7.65x10^8$	$1.06x10^8$
pH 2.0	0	0
pH 3.0	$4.08x10^7$	$1.18x10^7$
pH 4.0	$8.20x10^7$	$2.83x10^7$

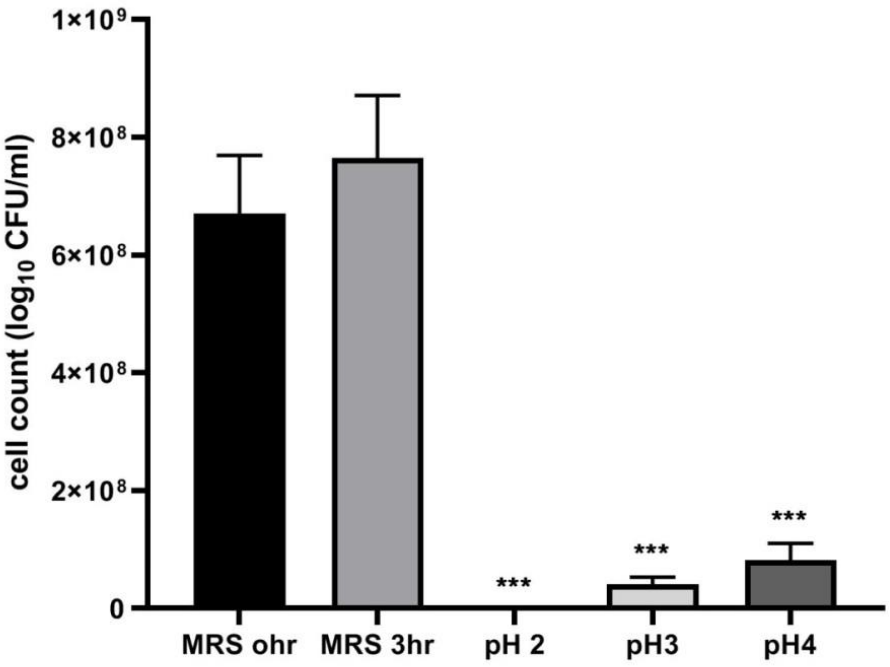


Figure 9 The survival of F23-5 isolate in different in pH values at 37°C for 3 h. Statistical analysis used one-way ANOVA (***p<0.001)

Table 10 The survival of F1-1 isolate in different concentration of bile at 37°C for 3 h.

% Bile	CFU/ml	SD
MRS o h.	$3.05x10^7$	$1.20x10^7$
MRS 3 h. (control) pH 6.5	$5.90x10^7$	$4.24x10^6$
0.3 % bile	$5.46x10^7$	$2.82x10^6$
0.8 % bile	$4.45x10^7$	$1.25x10^7$

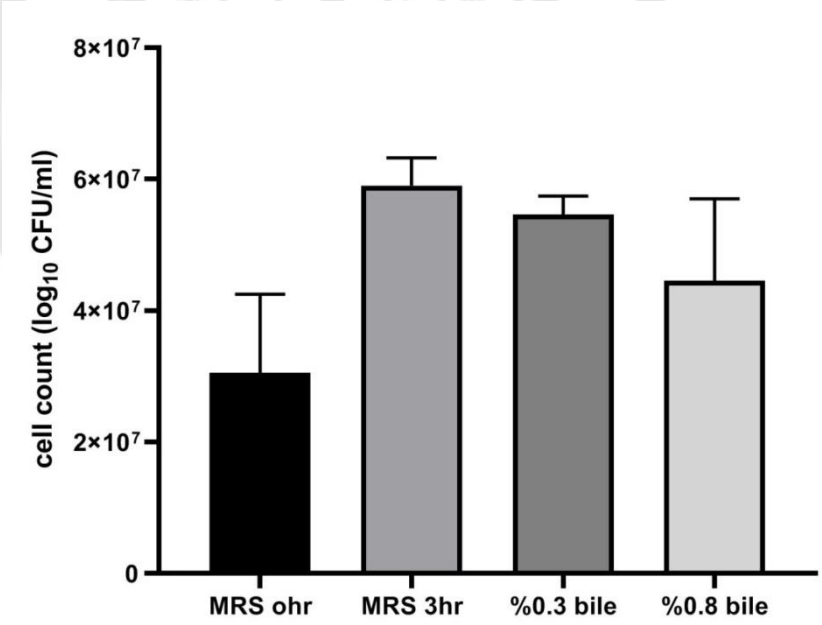
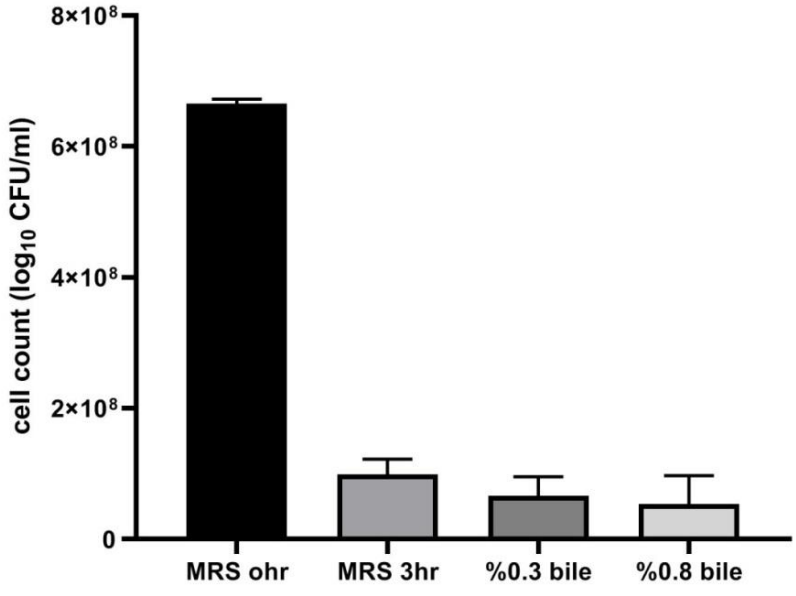


Figure 10 The survival of F1-1 isolate in different concentration of bile at 37°C for 3 h. Statistical analysis used one-way ANOVA

Table 11 The survival of F23-5 isolate in different concentration of bile at 37°C for 3 h.

% Bile	CFU/ml	SD
MRS o h.	$6.65x10^{8}$	$7.20x10^{6}$
MRS 3 h. (control) pH 6.5	$9.90x10^{7}$	$2.32x10^{7}$
0.3 % bile	$6.58x10^{7}$	$2.94x10^{7}$
0.8 % bile	$5.36x10^{7}$	$4.35x10^{7}$



The survival of F23-5 isolate in different concentration of bile

Figure 11 The survival of F23-5 isolate in different concentration of bile at 37°C for 3 h. Statistical analysis used one-way ANOVA

4.3.2 Phenotypic characteristics

Two isolate of F1-1 and F23-5 were identified on the basis of physiological and biochemical characteristics. Isolate F1-1 was rod-shaped, negative catalase test and gram-positive. This strain growth in 3% NaCl, 6.5% NaCl and at pH3, 4, 8, 9, 9.6 and growth at 20°C, 30°C and 37°C. Slime activity, gas production, nitrate reduction, amylase activity, arginine activity were exhibit negative.

F23-5 isolate was cocci-shaped, negative catalase test and gram-positive. This strain growth in 3% NaCl, 6.5% NaCl, and at pH3, 4, 8, 9, 9.6 and growth at 20°c, 30°, 37°c and 45°c. Arginine activity were exhibit positive and catalase test, Slime activity, Gas production, Nitrate reduction, Amylase activity were exhibited negative. (Table12.)

Carbohydrate fermentation test was used for classified of bacteria to fermented a specific carbohydrate. F1-1 isolate was exhibit produce acid from of 15 carbohydrate included arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, raffinose, ribose,s, Sucrose, trehalose and xylose.

F23-5 isolate was exhibit produce acid from of 13 carbohydrate include arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, ribose, salicin, sucrose and trehalose but did not produce acid from raffinose and xylose. (Table 13)

Table 12 Phenotypic characteristic of selected lactic acid bacteria

strain	NaCl (%)			Temperature (°C)				pH						Catalase test	Slime activity	Gas production	Nitrate reduction	Amylase activity	Arginine activity
	3	6.5	8	20	30	37	45	2	3	4	8	9	9.6						
F1-1	+	+	-	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-
F23-5	+	+	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	+

+ growth of bacteria, - not growth

Table 13 Acid production from carbohydate lactic acid bacterial strain

	Carbohydrate fermentation														
Strain	Arabinose	Cellobiose	Fructose	Galactose	Glucose	Lactose	Maltose	Mannitol	Mannose	Raffinose	Ribose	Salicin	Sucrose	Trehalose	Xylose
F1-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F23-5	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-

+ growth of bacteria, - not growth

4.3.3 Growth rate

The production media were incubated at 37°C for 36 h. under anaerobic condition using anaerobic jar. Measurements at OD600 was done every 3 h. Growth rate activity of 2 isolate of lactic acid bacteria were exhibit specific of growth rate. F1-1 isolate exhibit growth rate to lag phase at 0 to 2 h., log phase at 3 to 9 h. and stationary phase at 12 h.(Figure 12). F23-5 isolate exhibited growth rate to lag phase at 0 to 5 h., log phase at 6 to 9 h. and stationary phase at 15 h. (Figure 13).

Table 14 The OD growth rate of selected lactic acid bacteria

	F1-1		F23-5	
Time (h.)	OD600	pH	OD600	pH
0	0.20	6.35	0.2	6.34
3	0.456	5.150	0.203	5.6
6	0.831	4.870	0.29	5.44
9	1.737	4.630	0.402	5.28
12	1.903	4.400	0.532	5.09
15	2.143	4.340	0.578	4.97
18	2.170	4.200	0.616	4.96
21	2.307	4.200	0.666	4.85
24	2.390	4.200	0.657	4.76
27	2.510	4.170	0.655	4.71
30	2.487	4.180	0.651	4.62
33	2.690	4.200	0.63	4.43
36	2.700	4.160	0.622	4.38

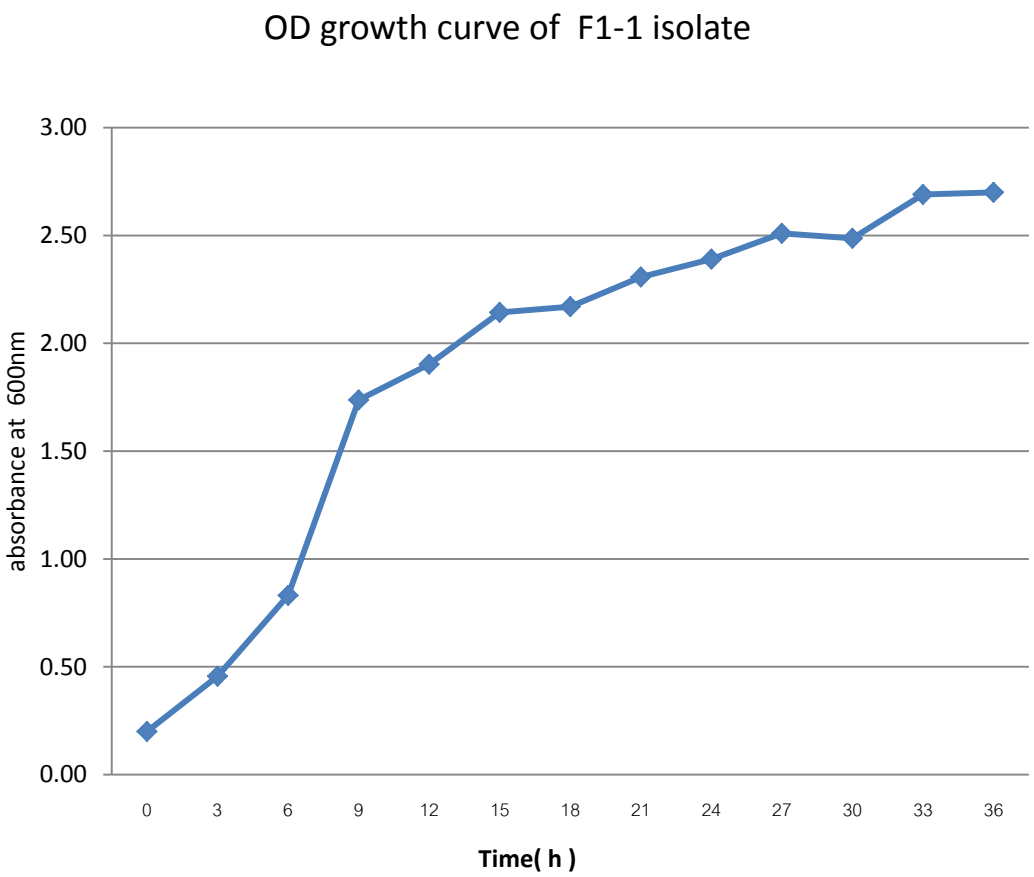


Figure 12 The OD growth curve of F1-1 isolate

This isolate exhibited growth rate at lag phase, log phase and stationary phase. Measurement at OD600 was every 3 h.

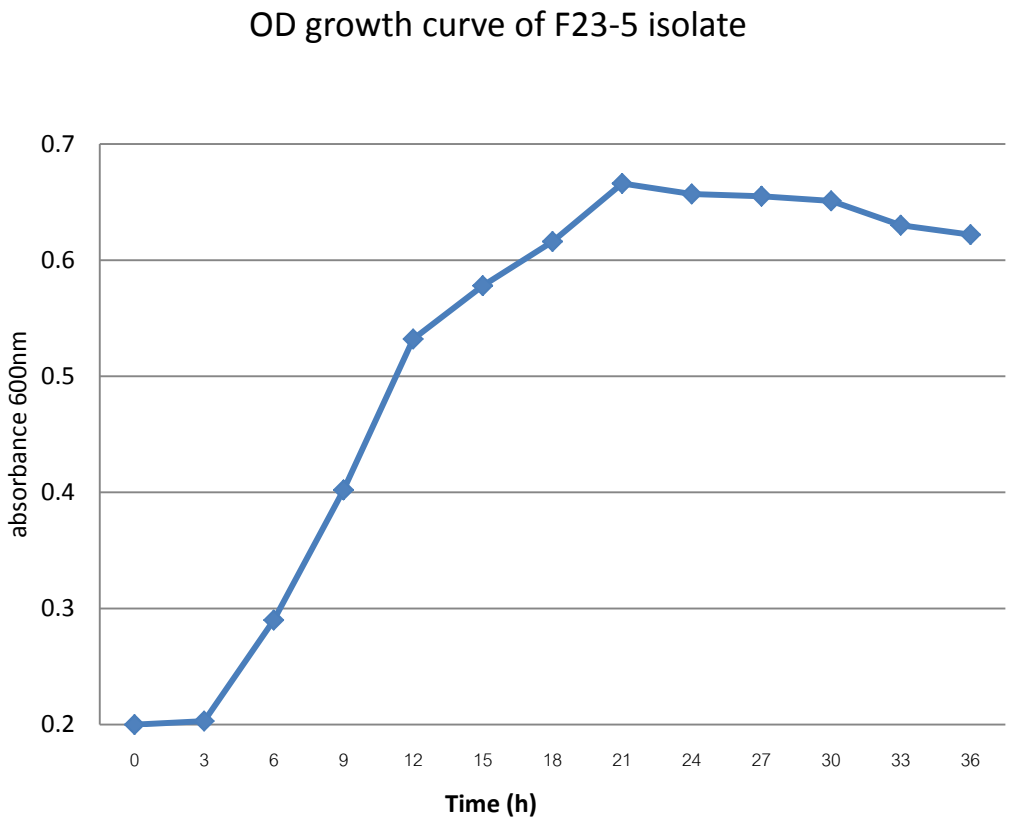


Figure 13 The OD growth curve of 23-5 isolate

This isolate exhibit growth rate at lag phase, log phase and stationary phase. Measurements at OD600 was every 3 h.

4.3.4 16S rDNA sequences

Screening of bile salt hydrolase activity and phenotypic, exhibit isolate of F1-1 and F23-5 were show strong bile salt hydrolase activity. To exactly confirm the identity of these isolates at species level, the 16S rDNA sequences was determined using BLAST software compared to EzTexon-e database. Phylogenetic tree analysis was performed to reveal the relationship between the representative isolates and the known reference strains. Based on the 16S DNA sequences, a representative F1-1 isolate was identified as *Lactobacillus pentosus* from 99.88% similarity of gene

sequence to *Lactobacillus pentosus* DSM 20314^{T} and 1360 bp (Table 15). This strin was detected in Sour meat (Nham nae).

F23-5 isolate was identified as *Enterococcus faecium* from 99.93% similarity of gene sequence to *Enterococcus faecium* CGMCC 1.2136^{T} and 1361 bp (Table 15). This strin was detected in Sour pork (Nham moo). The phylogenetic analysis was constructed with neighbor-joining as inferred by the neighbor-joining method. Bootstrap values (expressed as percentages of 1,000 replications) exhibit two cluster including *Lactobacillus* and *Enterococcus* (Figure 14)

Table 15 Species of lactic acid bacterial isolates

Isolate no.	Closest species	Similarity %	Length (bp)
F1-1	*Lactobacillus pentosus* DSM 20314^{T}	99.88	1360
F23-5	*Enterococcus faecium* CGMCC 1.2136^{T}	99.93	1361

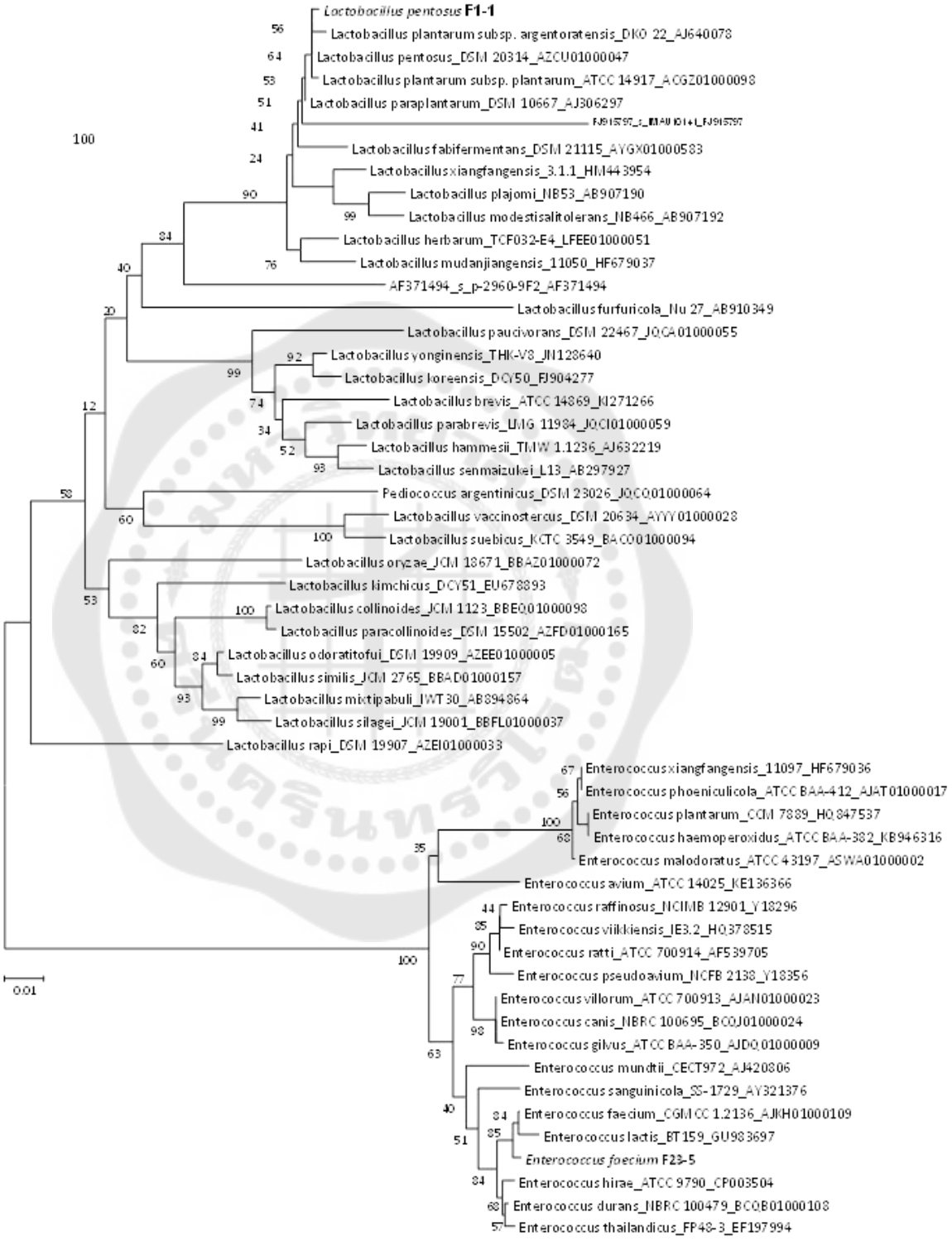


Figure 14 Phylogenetic tree based on 16S rDNA sequence

showing the relative position of *Lactobacillus* sp. and *Enterococcus* sp.

4.4 Animal study

4.4.1 Serum lipid profile

The mechanisms for cholesterol removal by probiotics have been proposed, such as the cholesterol assimilation into bacterial cell membranes, deconjugation of bile salts by bile salt hydrolase (BSH) and production of short-chain fatty acids (SCFAs) during growth of probiotics. Reducing cholesterol by probiotic lactic acid bacteria can be hypothesized by the fact that intake of Probiotic lactic acid bacteria increased the HDL-C concentration or/and resulted in lowering of the LDL-C level in the serum. The serum were examined the concentrations of Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C). Low-density lipoprotein cholesterol (LDL-C), and Triglycerides (TG).

Rats fed with the High-Fat diet group exhibit higher total cholesterol levels compared with the normal group, while the groups that received probiotic lactic acid bacteria F23-5 isolate solution (1 ml) exhibited total cholesterol level 48.2 mg/dl had significantly lower ($P < 0.001$) total cholesterol levels compare with rat fed high-fat group was 92 mg/dl as shown in Figure 15.

F23-5 isolate show that higher High-density lipoprotein cholesterol compare with control group. The High-Fat diet +probiotic LAB F1-1, probiotic LAB F1-1 and probiotic LAB 23-5 group were not significant. As exhibited in Figure 16, The High-Fat diet +probiotic LAB F23-5 group had high high-density lipoprotein cholesterol compare with High-Fat diet group but not significantly. As exhibited in Figure 17, show no difference of low-density lipoprotein cholesterol. As exhibited in Figure 18, all group show high level of triglycerides (mg/dl) in rats.

Table 16 Serum cholesterol levels of rats in different diet-fed groups.

Group	Total cholesterol (mg/dl)	SD
Normal group	69.2	12.62
High-Fat diet group	92	4.062
High-Fat diet +probiotic LAB F1-1	75.4	10.92
High-Fat diet +probiotic LAB F23-5	48.2	6.94
Probiotic LAB F1-1	73.8	9.26
Probiotic LAB 23-5	69.4	8.93

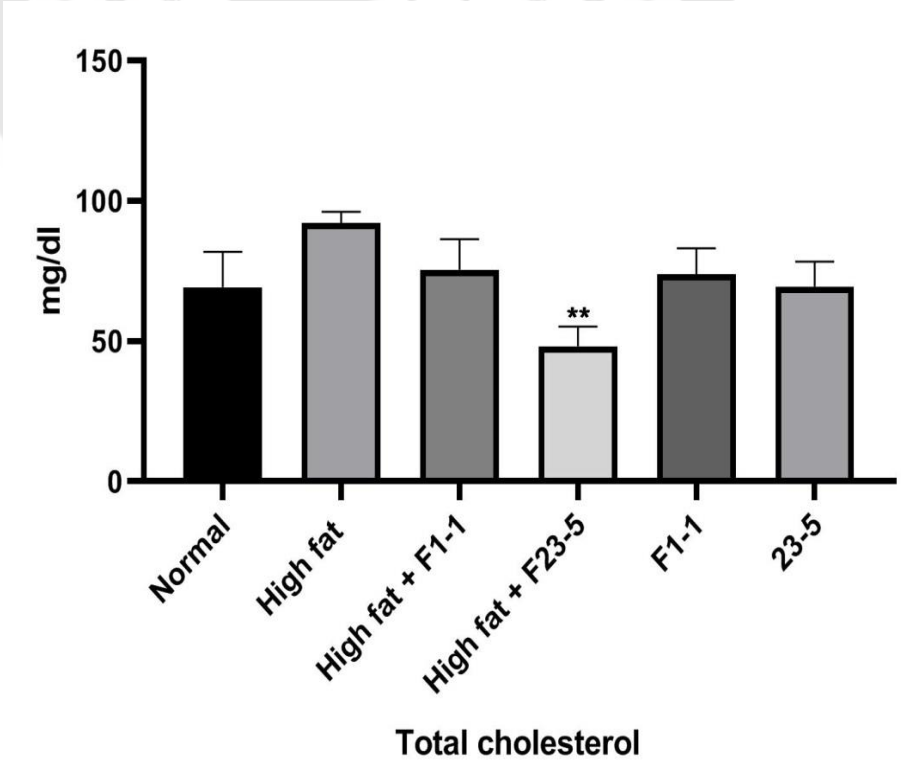


Figure 15 Total cholesterol (mg/dl) in animal group

Control group, high-fat diet , high-fat diet with Probiotic F1-1 and F23-5 and Probiotic LAB. Statistical analysis used one-way ANOVA (**p<0.001)

Table 17 Serum cholesterol levels of rats in different diet-fed groups.

Group	high-density lipoprotein cholesterol (mg/dl)	SD
Normal group	49	9.54
High-Fat diet group	46.2	8.87
High-Fat diet +probiotic LAB F1-1	46.6	4.83
High-Fat diet +probiotic LAB F23-5	58.6	12.76
Probiotic LAB F1-1	47	13.41
Probiotic LAB 23-5	47.6	6.99

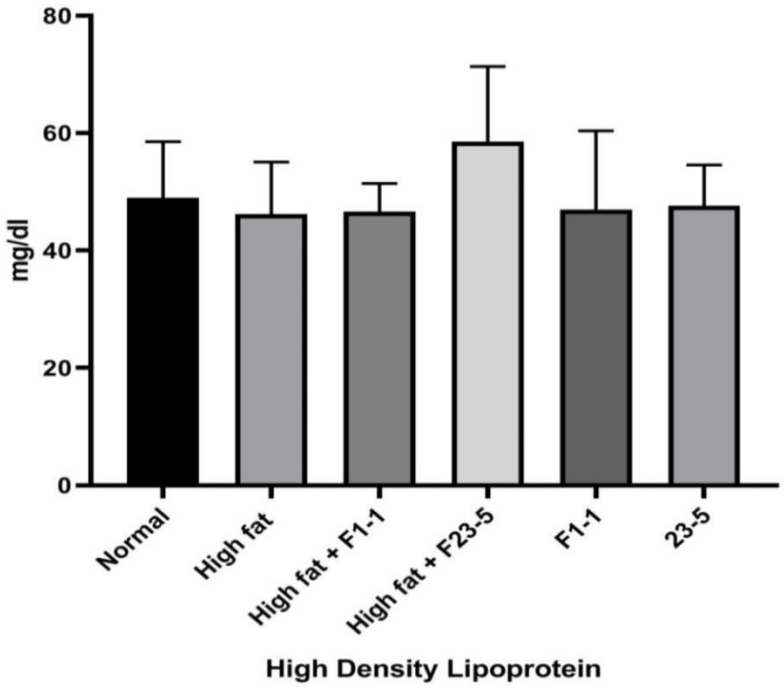


Figure 16 High-density lipoprotein cholesterol (mg/dl) in animal group Control group, high-fat diet , high-fat diet with Probiotic F1-1 and F23-5 and Probiotic LAB. Statistical analysis used one-way ANOVA

Table 18 Serum cholesterol levels of rats in different diet-fed groups.

Group	Low-density lipoprotein cholesterol (mg/dl)	SD
Normal group	4.8	0.84
High-Fat diet group	5	0.71
High-Fat diet +probiotic LAB F1-1	4	0
High-Fat diet +probiotic LAB F23-5	5.2	1.10
Probiotic LAB F1-1	4.6	0.55
Probiotic LAB 23-5	4.4	0.55

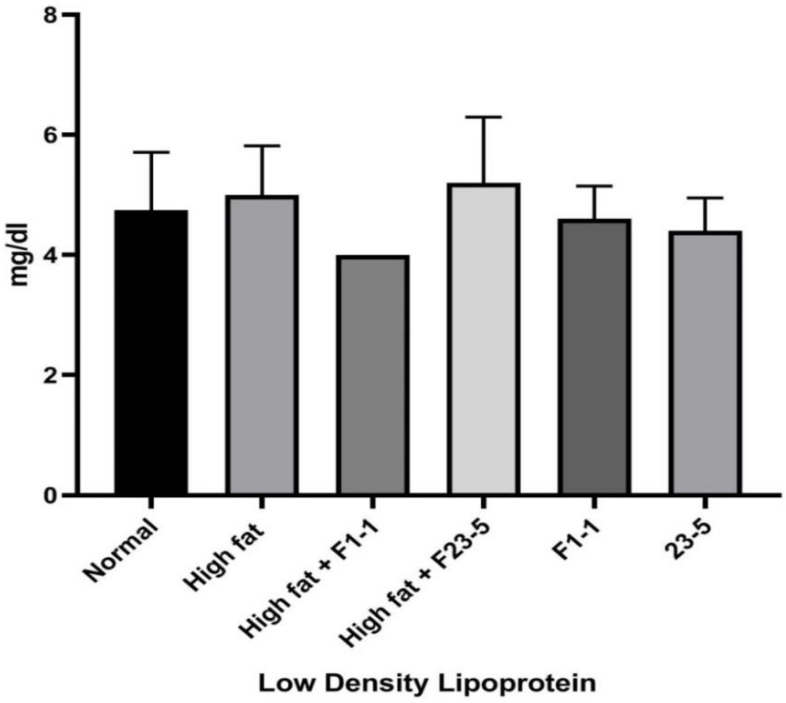


Figure 17 High-density lipoprotein cholesterol (mg/dl) in animal group Control group, high-fat diet , high-fat diet with Probiotic F1-1 and F23-5 and Probiotic LAB. Statistical analysis used one-way ANOVA

Table 19 Serum cholesterol levels of rats of different diet-fed groups.

Group	Triglycerides (mg/dl)	SD
Normal group	207.6	46.75
High-Fat diet group	252	81.0
High-Fat diet +probiotic LAB F1-1	438.2	147.96
High-Fat diet +probiotic LAB F23-5	300	86.25
Probiotic LAB F1-1	258.2	138.19
Probiotic LAB 23-5	269	140.62

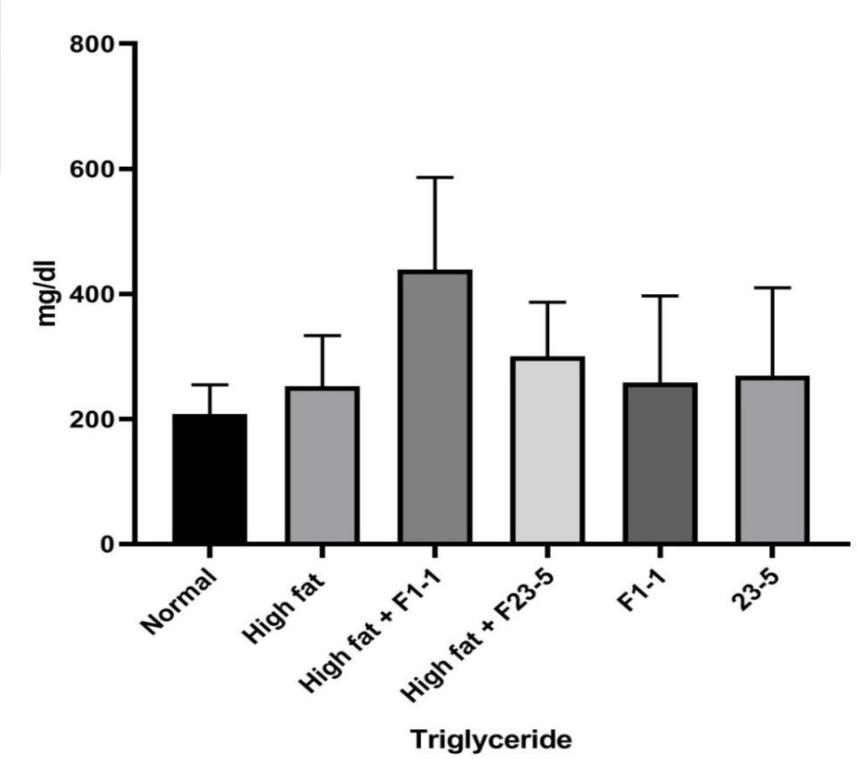


Figure 18 Triglycerides (mg/dl) in animal group

Control group, high-fat diet , high-fat diet with Probiotic F1-1 and F23-5 and Probiotic LAB. Statistical analysis used one-way ANOVA

4.4.2 Intestinal microbiota analysis

Fecal samples were collected on days 0 (pre-treatment) and 28 (after-treatment). Each serial dilution was transferred to the Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and Bifidobacterium Selective Media (BSM) by spread plate technique and incubated at 37°C for 48 hr under anaerobic condition.

The EMB selective media were showed 4 colonies on media plate included black, punctiform, pink, circular, pink, punctiform and purple,circular. MRS were showed 4 shaped colonies on media plate white, circular, white, circular, raised, white, circular and white, undulate. BSM were 2 colony shape on media plate included white, circular and white, punctiform.

Normal group were exhibit bacterial colony count on EMB plate not much difference between pre-treatment and after- treatment. Bacterial colony count on MRS and BSM (Table19) were not difference in colony count between pre-treatment and after-treatment.

High-fat diet group were exhibited colony count on EMB media pre-treatment (not fed high fat) fond 4 shape of colony and after-treatment (after fed high fat) had significant decrease 3 shape of colony on EMB media (Table 19). Colony count on MRS was not difference between pre-treatment and after- treatment, colony count BSM media exhibited after-treatment were high of colony count compare pre-treatment.

High-fat diet +probiotic LAB F1-1 group were exhibited bacterial colony count on EMB media were not different between per-treatment and after-treatment, colony count on MRS media have decrease 2 shape of colony form 4 shape colony. BSM media exhibited after-treat were significant induce colony count on media.

High-fat diet +probiotic LAB F23-5 groups,after-treatment reduced colony count on EMB and MRS media compare to per-treatment. BSM media exhibit not different between per-treatment and after-treatment.

Probiotic LAB F1-1 group after-treat exhibited decreased colony count on EMB and MRS media compare to pre-treatment, BSM media exhibit after-treatment were significant induce colony count on media.

Probiotic LAB 23-5 group was indicated that after-treatment decreased bacterial colony count on EMB and BSM media compare to pre-treatment and MRS media exhibited not difference bacterial colony count between pre-treatment and after-treatment.

Table 20 Viable cell count (CFU/ml) on selective media

	Selective media	Colony characteristics	Normal group	High-Fat diet group	High-Fat diet +probiotic LAB F1-1 group	High-Fat diet +probiotic LAB F23-5 group	Probiotic LAB F1-1 group	Probiotic LAB 23-5 group
Pre-treatment	EMB	Black, punctiform	$9.4x10^6$	$4.7x10^6$	0	$7.8x10^6$	$1.14x10^7$	$5.6x10^7$
		Pink, circular	$4.0x10^6$	$4.6x10^6$	$4.3x10^5$	$2.8x10^6$	$8.0x10^5$	$1.37x10^8$
		Pink, punctiform	$4.1x10^6$	$6.5x10^6$	$3.5x10^5$	$1.33x10^7$	$3.9x10^6$	$2.3x10^7$
		Purple, circular	$2.2x10^6$	$3.5x10^6$	$1.7x10^5$	0	0	0
	MRS	White, circular+â	$5.1x10^7$	$5.2x10^7$	$2.5x10^6$	$1.10x10^7$	$6.9x10^7$	$1.48x10^8$
		White, circular,raised	$1.06x10^8$	$4.1x10^7$	$2.2x10^6$	$5.4x10^6$	$1.9x10^7$	0
		White, circular	$4.9x10^7$	$4.1x10^7$	$2.4x10^6$	$1.64x10^7$	$1.21x10^8$	$5.1x10^7$
		White, undulate	$1.02x10^8$	$6.7x10^7$	$1.23x10^7$	$7.9x10^6$	$7.9x10^7$	$5.5x10^7$
	BSM	White, circular	$1.27x10^7$	$5.5x10^6$	$6.6x10^5$	$4.4x10^6$	$9.5x10^6$	$1.45x10^8$
		White, punctiform	$7.6x10^6$	$4.3x10^6$	$1.8x10^5$	$2.1x10^6$	$4.7x10^6$	$1.8x10^7$
After-treatment	EMB	Black, punctiform	$3.0x10^7$	$7.0x10^5$	$8.2x10^5$	$1.45x10^8$	$3.8x10^5$	$7.1x10^6$
		Pink, circular	$3.4x10^7$	0	$3.0x10^5$	0	0	$2.5x10^6$
		Pink, punctiform	$2.7x10^7$	0	$4.4x10^5$	$1.33x10^8$	$2.5x10^5$	$1.5x10^6$
		Purple, circular	$9.0x10^6$	0	$3.3x10^5$	0	0	0
	MRS	White, circular+â	$6.1x10^6$	0	$5.0x10^6$	$5.1x10^6$	$6.9x10^7$	$3.7x10^7$
		White, circular,raised	$5.1x10^6$	$1.97x10^7$	$5.7x10^6$	$3.0x10^6$	$6.8x10^7$	0
		White, circular	$1.33x10^7$	$1.87x10^7$	0	$9.2x10^6$	0	$4.7x10^7$
		White, undulate	$5.4x10^6$	$2.09x10^7$	0	0	0	$1.59x10^8$
	BSM	White, circular	$3.8x10^7$	$1.92x10^7$	$1.33x10^7$	$3.5x10^7$	$1.03x10^8$	$4.51x10^7$
		White, punctiform	$1.04x10^8$	$2.2x10^7$	$1.57x10^7$	$7.3x10^7$	$7.9x10^7$	$4.18x10^7$

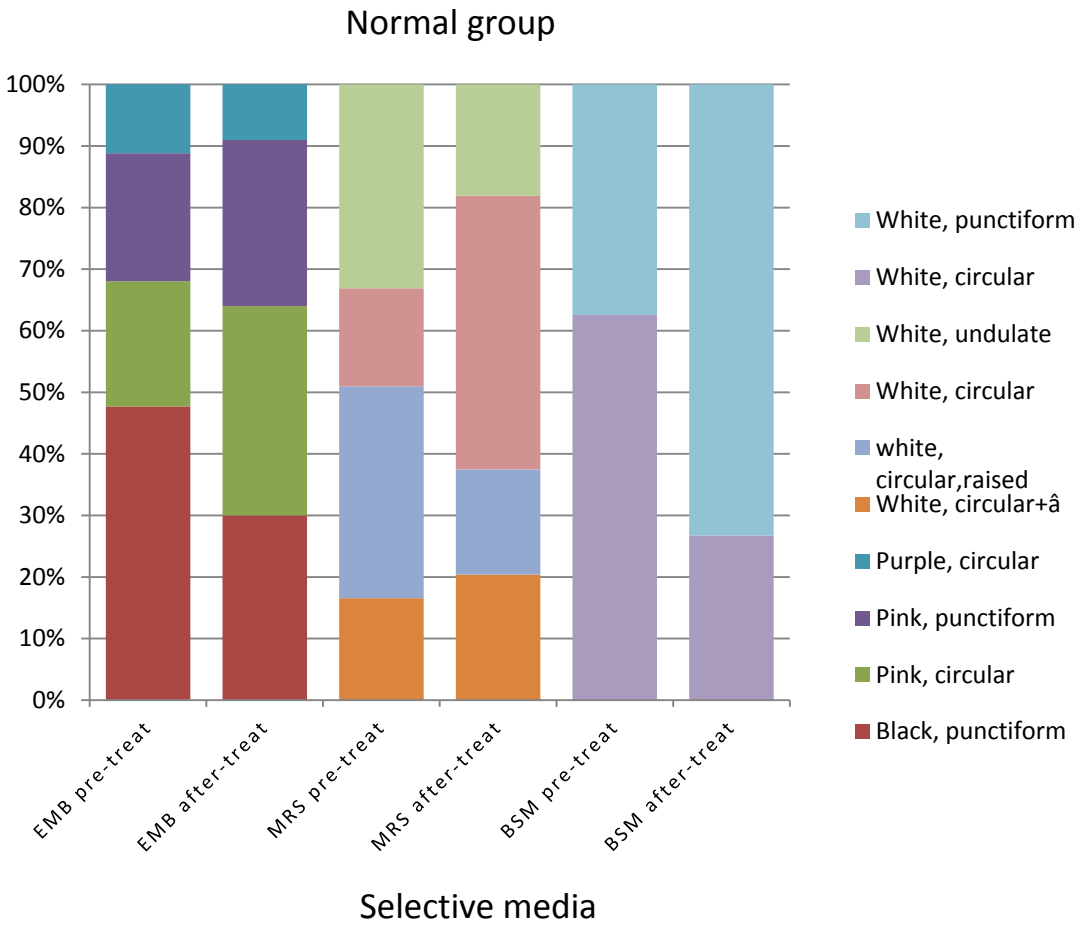


Figure 19 Intestinal microbiota analysis in normal group collected on day 0 (pre-treat) and 28 (after-treat) by Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and Bifidobacterium Selective Media (BSM)

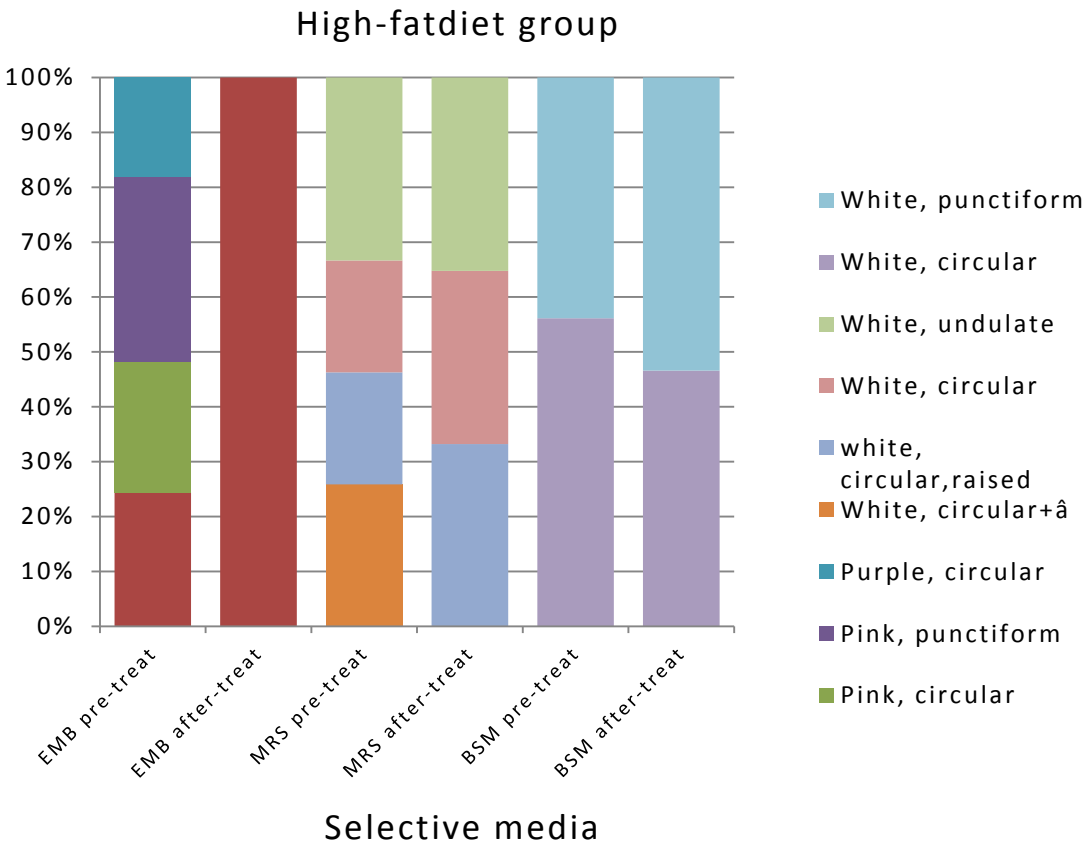


Figure 20 Intestinal microbiota analysis in high-fat group collected on day 0 (pre-treat) and 28 (after-treat) by Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and Bifidobacterium Selective Media (BSM)

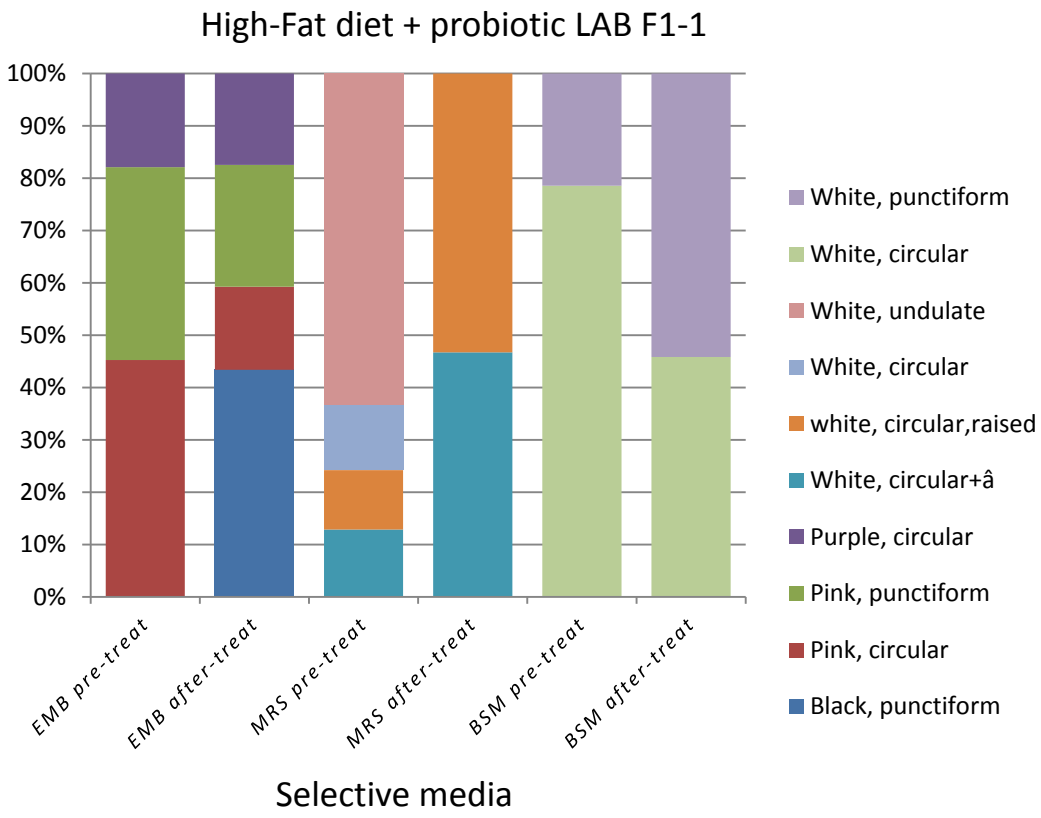


Figure 21 Intestinal microbiota analysis in high-fat diet + probiotic LAB F1-1 group collected on day 0 (pre-treat) and 28 (after-treat) by Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and Bifidobacterium Selective Media (BSM)

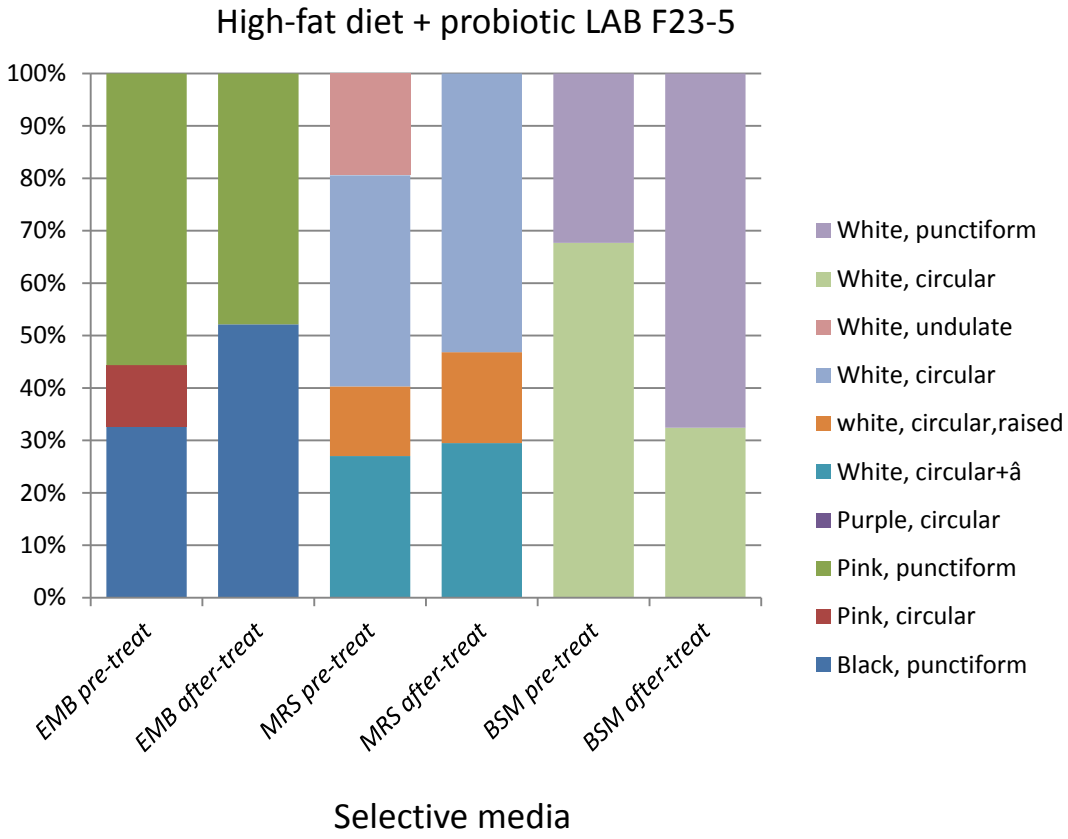


Figure 22 Intestinal microbiota analysis in high-fat+ F23-5 group collected on day 0 (pre-treat) and 28 (after-treat) by Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and Bifidobacterium Selective Media (BSM)

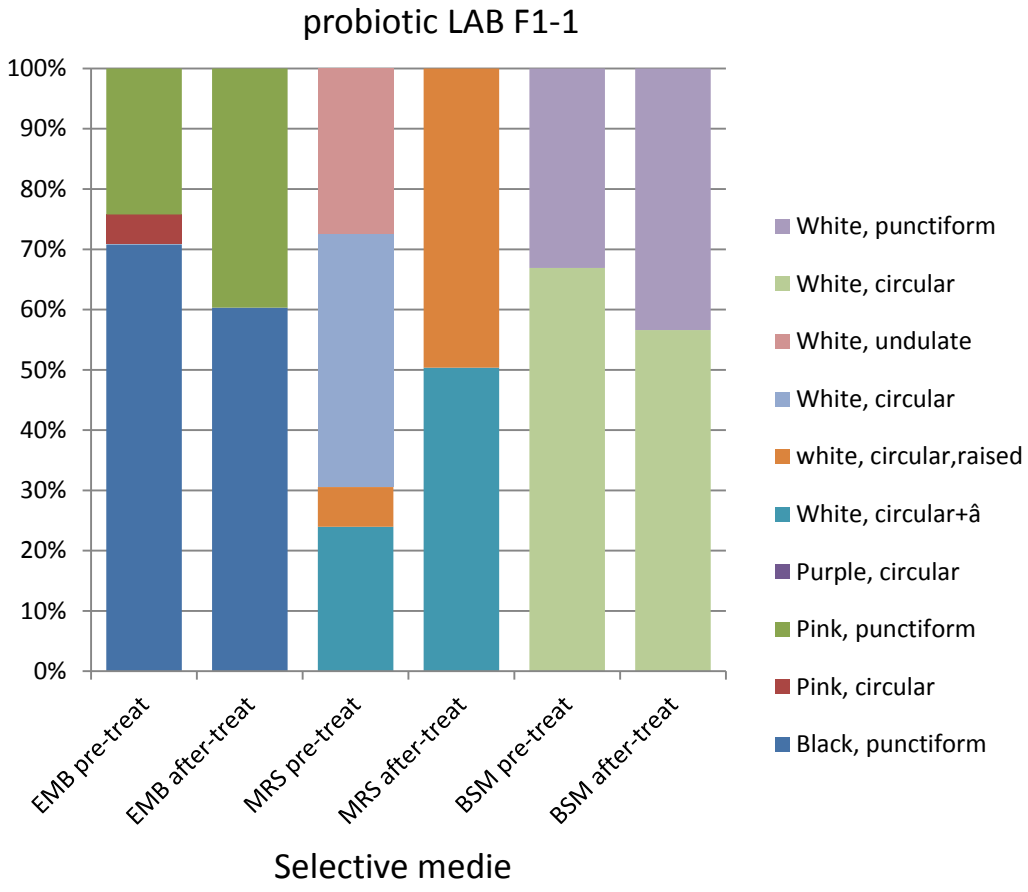


Figure 23 Intestinal microbiota analysis in F1-1 LAB group collected on day 0 (pre-treat) and 28 (after-treat) by Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and Bifidobacterium Selective Media (BSM)

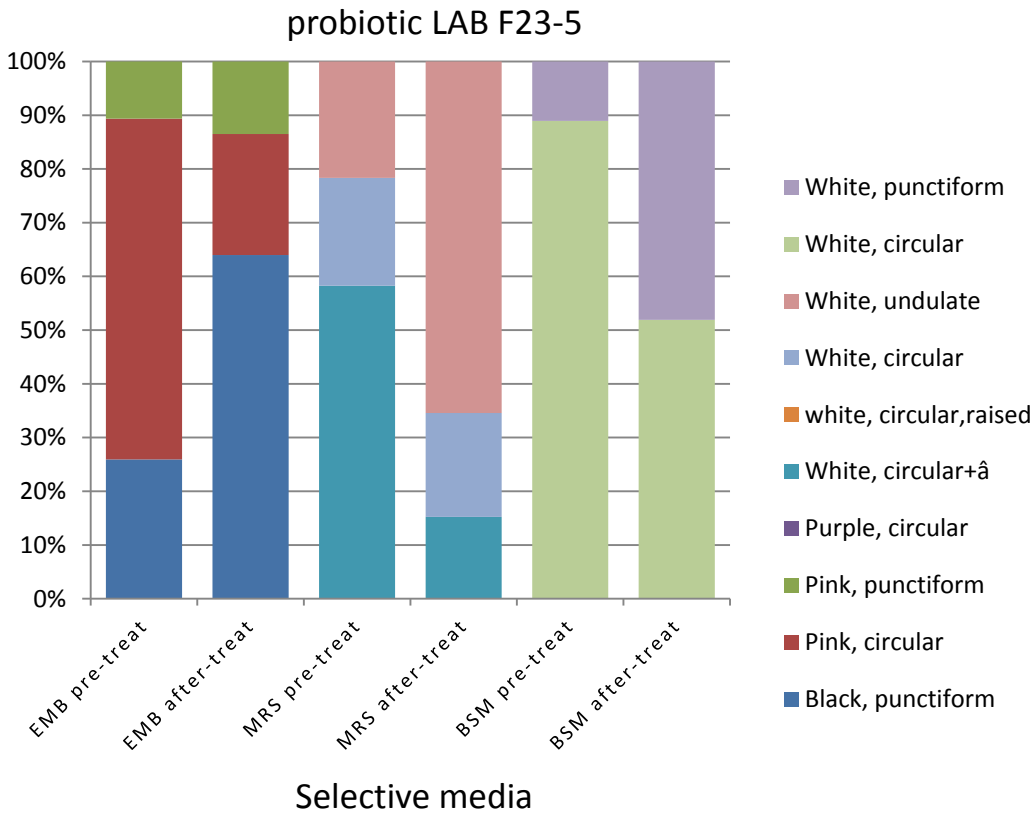


Figure 24 Intestinal microbiota analysis in F23-5 LAB group collected on day 0 (pre-treat) and 28 (after-treat) by Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and Bifidobacterium Selective Media (BSM)

4.5 The quality parameters of probiotic lactic acid bacteria in fermented milk

F23-5 isolate was reduced total cholesterol level in rat fed high-fat diet., this strain was selected to study the fermentation in milk product. Duration of milk fermentation by probiotic was 18 h. for pH value 4.61. After fermentation, the fermented milk was storage at 4°C for 28 days.

4.5.1 Viability of probiotics in fermented milk product

Viable counts of F23-5 isolate was counted and displayed as cell numbers by log10 of colonies grown on MRS agar. Fermented milk sample decreased with storage time (28 days) from $6.53x10^{12}$ CFU/mL to $1.55x10^{8}$ CFU/ml. Viable counts were higher than 10^{6} CFU/mL until the 28th day of storage (Table 21). For exerting potential benefits to the consumer required by Food and Drug Administration, Thailand.

Table 21 Viability of probiotics in milk product using starter probiotic cultures

Storage period (day)	CFU/ml	SD
1	$6.53x10^{12}$	$2.31 x10^{12}$
7	$1.82x10^{12}$	$1.06 x10^{12}$
14	$6.26 x10^{8}$	$5.12 x10^{8}$
21	$5.58 x10^{8}$	$1.39 x10^{8}$
28	$1.55x10^{8}$	$1.30 x10^{8}$

4.5.2 pH value

The pH values of the fermented milk products were determined using a pH meter. The pH value in fermented milk sample exhibit pH value between 4.61 to 4.93 and the pH value decreased 4.61 at 1 day, 4.49 at 7 day, In contrast, the pH value increase to 5.35 at 14 day, 5.16 day, and 4.93 at 28 day as show as Table 22. The pH drop within the 7 day of storage, as the bacterial produced higher amount of lactic acid through lactose hydrolysis was especially fast during the initial period.

Table 22 pH value in milk product using starter probiotic cultures using starter probiotic cultures during storage at 4°C for 28 days

Storage period (day)	pH	SD
1	4.61	0.006
7	4.49	0.060
14	5.35	0.03
21	5.16	0.06
28	4.93	0.09

4.5.3 Apparent viscosity

Viscosity of fermented milk products were using a Brookfield Viscometer with adjusted rotational speed of 0.5 rpm. The viscosity were read at the 15th second during the measurement. The apparent viscosity of milk product sample with F23-5 isolate as the storage period having higher apparent viscosity. Apparent viscosity were 1.23 Pa.s at 1day, 1.32 Pa.s at 7 day, 1.35 Pa.s at 14day, 1.32 Pa.s at 21 day and 1.27 Pa.s at 28 day respectively.

Table 23 Viscosity characteristics at shear rate = 22 s^{-1} of milk product using starter probiotic culture during storage at 4°C for 28 days

Storage period (day)	Apparent viscosity (Pa.s)
1	1.23
7	1.32
14	1.35
21	1.32
28	1.27

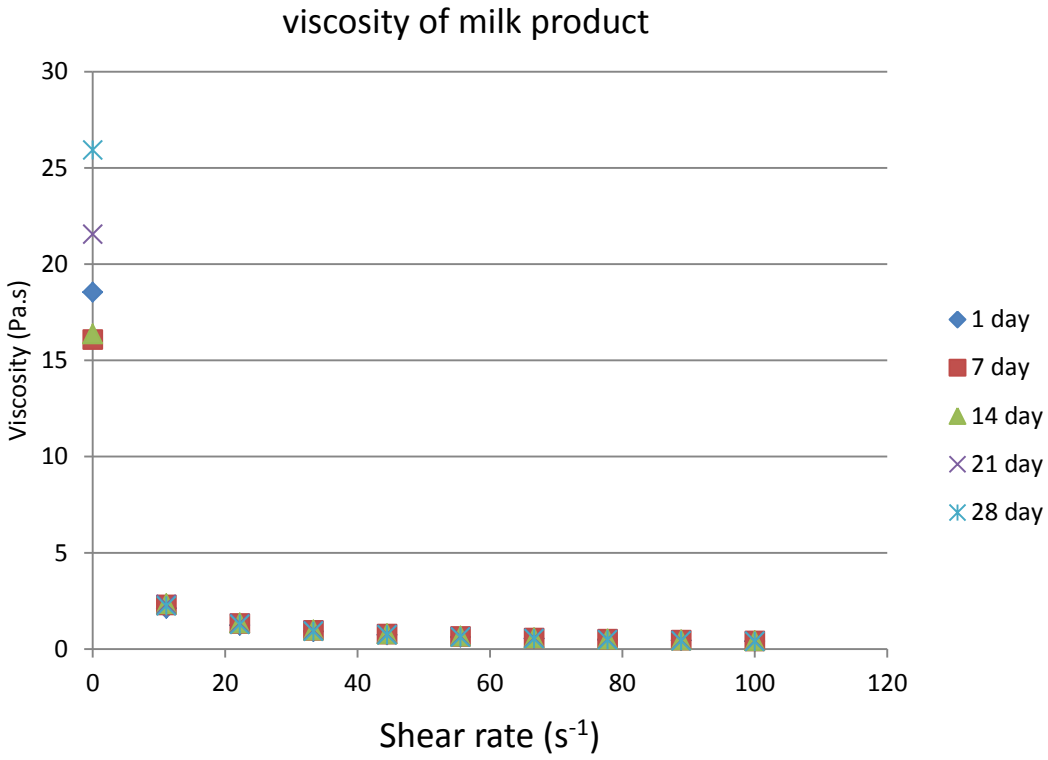


Figure 25 Viscosity characteristics of milk product fermented by probiotic lactic acid bacteria F23-5 during storage at 4°C for 28 days

4.5.4 Rheological

The rheological of fermented milk products were using Dynamic oscillatory measurements were performed with a HAAKE RheoStress1Rheometer (Thermo Sciencetific, Waltham, MA, USA). Rheological of fermented milk product sample with F23-5 isolate as the storage period having higher G'(Pa). G' were 137.42 Pa at 1 day, 152.08 Pa at 7 day, 172.22 Pa at 14 day, 179.66 Pa at 12 day and 169.23 Pa at 28 day respectively.

Table 24 Rheological characteristics in milk product using starter probiotic cultures storage modulus and loss tangent at frequency of 1 Hz. during storage at 4°C for 28 days.

Storage period (day)	G' (Pa)	Loss tangent
1	137.42	0.27
7	152.08	0.41
14	172.22	0.46
21	179.66	0.2
28	169.23	0.42

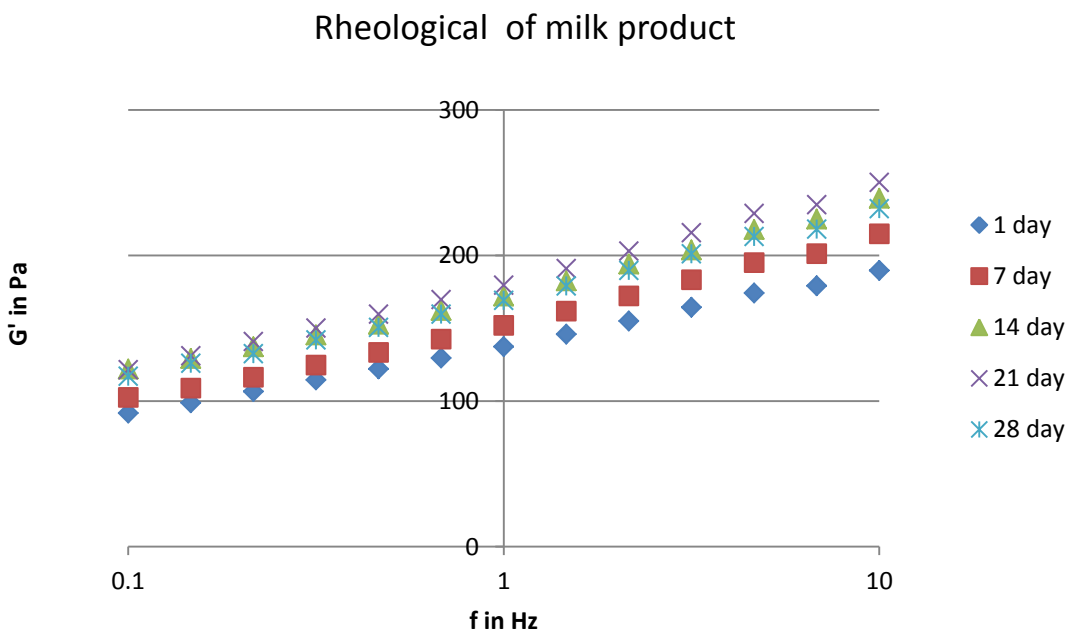


Figure 26 Rheological characteristics of milk product fermented by probiotic lactic acid bacteria F23-5 during storage at 4°C for 28 days

4.5.5 Texture characteristics

Texture of fermented milk was determined using a compression test carried out with a TA.XTplus Texture Analyser (Stable Micro Systems, Surrey, UK). The textures properties of the fermented milk sample exhibit to the highest firmness 51.15 g to 69.55g. Firmness of fermented milk sample perennially increase to storage period were 51.150g at 1 day , 56.7g at 7day, 59.433g at 14day, 68.183g at 21 day and 69.55g at 28 day respectively. The fermented milk by F23-5 isolate had the highest content of 10% milk powder cause increase highest firmness and the best texture.

Table 25 Texture characteristics in milk product using starter probiotic cultures during storage at 4°C for 28 days

Storage period (day)	Firmness(g)	SD
1	51.15	4.56
7	56.70	4.96
14	59.433	5.21
21	68.183	5.67
28	69.55	7.45

4.5.6 Syneresis

Syneresis of fermented milk product was indicated in percentage (%) of syneresis which calculated by equation (% Syneresis = Weight of whey (g)/total weight of milk (g) ×100). Syneresis regression equation of fermented milk product sample with strain F23-5 as exhibit Percent (%) syneresis was lower of all storage time period at 1 to 21 day were 0.00 % seneresis and after 14 days were a little increase (%) syneresis in fermented milk sample.

Table 26 Syneresis characteristics in milk product using starter probiotic cultures during storage at 4°C for 28 days

Storage period (day)	Synersis (%)	SD
1	0.00	0.00
7	0.00	0.00
14	0.65	0.00
21	0.85	0.19
28	1.09	0.17

CHAPTER V
DISCUSSION

In this study, preliminary isolation of lactic acid bacteria including lactic acid production, cell morphology features, catalase test and Gram-staining were performed. Seventy five lactic acid bacteria were isolated from traditional fermented Thai foods. All isolates were lactic acid production, Gram-positive, catalase-negative, and rod- or cocci-shaped bacteria (Table 6). The traditional fermented Thai food samples for isolation have a generally high lactic acid bacteria count which are the good isolation sources of lactic acid bacteria.

Hypercholesterolemia is a main risk factor for the progress of coronary heart disease. Thus, reducing the cholesterol level of serum is essential to prevent the disease [100]. Probiotics have been attributed to some mechanisms, such as the interpolation of cholesterol into the cell membrane, cholesterol assimilation, and adhesion of cholesterol on the cell surface[101]. As a result, new bile acids from the cholesterol begin to synthesize. Therefore, the total concentration of cholesterol reduces in the body. *L. plantarum* DP4, DP3, DP14, *L. casei* DP29, *L. brevis* DP41 and DP44 exhibited hypocholesteromic effect and could possibly be applied to prevent hypercholesterolemia[102]. Cholesterol-lowering ability and BSH activity in vitro were critical parameters for screening probiotics possessing potential activity (Tsai et al., 2014 and Song et al., 2015). All seventy five lactic acid bacteria were screened for bile salt hydrolase (BSH) activity by direct plate assay. The results were showed in table 7, two isolates of F1-1and F23-5 have the highest bile salt hydrolase activity. The presence of bile salt hydrolase (BSH) in probiotics, which also helps to reduce the blood cholesterol level of the host [98]. BSH activity can be detected in all some species such as lactobacilli and bifidobacteria strains [99]. BSH is deconjugation of bile acid, which makes the bile salt less soluble and be excreted out as free bile acid via faces. This will reduce the cholesterol in serum and increase synthesis to replace the lost bile acid.

The important properties of probiotic bacteria are the ability to survive in the acidic and bile tolerance in the gastrointestinal tract. Acid and bile tolerance is one of

the most crucial properties used to select potentially probiotic bacteria strains [103]. The pH values of 2.0, 3.0 and 4.0 and bile salt concentration at 0.3% and 0.8% compared to MRS media control (pH 6.5). F1-1 isolate was tolerated and able to survives at pH 3.0 and pH 4.0 with the number $4.153x10^8$ CFU/ml and $7.675x10^8$ CFU/ml respectively. F23-5 isolate exhibited ability to survives at pH 3.0 and pH 4.0 with the number $4.153x10^7$ and $7.675x10^7$ CFU/ml respectively. F1-1 and F23-5 isolates did not survive at pH 2.0 as compared to MRS control at pH 6.5, pH 2 is a strong acidic may affect to cell membran of bacteria were not abiliyt to survive. All isolate were tolerance in pH values of 3.0 and 4.0 and bile salt concentration at 0.3% and 0.8%, Evidence indicates that ability to adhere to the intestinal epithelium cell have been considered as one of the selection criteria for probiotic strains. Probiotic must survive under severe condition in the stomach with low pH and resist bile salt to adhere to the intestinal.

Two isolate of F1-1 and F23-5 were the highest bile salt hydrolase activity was isolated from traditional fermented Thai food of Bangkok Thailand. These isolates were identified on the basis of physiological and biochemical characteristics presented in Tables 12 and 13. The result of phenotype (biochemical test and carbohydrate test) show that conformable lactic acid bacteria in general characteristics [104]. Growth rate of cell bacteria show as different at time to growth cell bacteria. The result show that two isolate of lactic acid bacteria to growth rate activity exhibit specific of time rate to growth cell bacteria such as lag phase, log phase and stationary phase (figure 11-figure 12). Bacterial growth rates during the phase of exponential growth, under standard nutritional conditions included culture medium, temperature, pH and etc.[105]

These strain were further identified on the basis of 16S rDNA sequencing as F1-1 isolate showed 99.88% similarity to *Lactobacillus pentosus* DSM 20314^T and 1360 bp (Table 15), this strain was detected in sour meat (Nham nae) collected bangkok Thailand. Montet et al.(2014) report that *Lactobacillus pentosus* was the predomaint laactic acid bacteria associated with fermented vegetables and fermented meat.

F23-3 isolate clearly showed 99.93% similarity to *Enterococcus faecium* CGMCC 1.2136^T and 1361 bp (Table 15), this strain was detected in sour pork (Nham

moo) collected bangkok Thailand. Maki (2014) report that *Enterococcus faecium* isolated from Khanom-jeen product aqnd could be found in many meat fermented. Phylogenetic relationship between lactic acid bacteria were constructed based on the 16S rDNA sequences from evolutionary distances by the neighbor-joining method, The phylogenetic analysis was constructed with neighbor-joining exhibit two cluster including *lactobacillus* and *Enterococcus.* (Figure 13).

Hypercholesterolemia is considered a major risk factor for the development of coronary heart disease, and although pharmacologic agents are available to treat this condition.[106] These cholesterol-lowering effects mechanisms include BSH activity, assimilation of cholesterol by the bacteria, binding of cholesterol to the bacterial cell walls, or physiological actions of the end products of short-chain fatty acid fermentation.[107] In this study we select 2 isolate of F1-1 and F23-5 which were show higher bile salt hydrolase activity were selected to test in animal model study, The serum were examined the concentrations of serum Total cholesterol (TC), High-density lipoprotein cholesterol (HDL-C). Low-density lipoprotein cholesterol (LDL-C), and Triglycerides (TG). The results show that rats fed with the high-Fat diet group exhibit higher total cholesterol levels compared with the normal group, while the groups that received probiotic lactic acid bacteria LAB F23-5 solution (1 ml) exhibited total cholesterol level 48.2 mg/dl had significantly lower ($P < 0.001$) total cholesterol levels compare with rat fed high-fat group was 92 mg/dl as shown in Figure 14. F23-5 strain show that higher High-density lipoprotein cholesterol compare with control group. Hypocholesterolemic effects of probiotic mixture on diet-Induced hypercholesterolemic rats by Shang-Jin Kim, this of probiotic mixture of two lactobacilli and three bifidobacteria has the potential to reduce serum total cholesterol, triglycerides, and LDL-cholesterol levels in hypercholesterolemic rats[81]. *E. faecium* WEFA23 was found to have potential for improvement of MS symptoms, including obesity and hyperlipidemia in HFD rats, these stain showed desirable probiotic properties of cholesterol removal, BSH activity, and adhesion ability.[108]

Fecal samples are often used to investigate the intestinal microbiota because they are easily collected and not invasive to the host.[109] Fecal sample were collected on days 0(pre-treat) and 28(after-treat) Each serial dilution were transferred to the Eosin methylene blue (EMB), De Man Rogosa Shape (MRS) and Bifidobacterium Selective Media (BSM) by Spread plate technique and incubated at 37°C for 48 h under anaerobic condition.

In this study, the result of microbiota show as some group bacteria form whole bacteria count in intestine tract in rat. Viable cell count on selective media (table 20) show the different colony characteristic, After-treated in animal study group compare with pre-treated group; high-fat diet, high-fat diet +probiotic LAB F23-5, probiotic LAB F1-1 and probiotic LAB 23-5. The result indicated that after-treated have decreased bacteria count on EMB media plat compare with pre-treated group. Normal, high-fat diet and probiotic LAB 23-5 groups revealed that bacteria count not different on MRS media between pre-treated and after-treated, high-fat diet +probiotic LAB F1-1, high-fat diet +probiotic LAB F23-5 and probiotic LAB F1-1 groups were decreased bacteria count on MRS media. High-fat diet, high-fat diet +probiotic LAB F1-1 and probiotic LAB F1-1 groups were induced bacteria count on BSM media, normal, high-fat diet +probiotic LAB F23-5 and probiotic LAB 23-5 groups were not different bacteria count on BSM media after-treatment.

F23-5 isolate exhibit decrease total cholesterol level in rat fed high fat, there for select to use fermentation in milk product. This milk product by F23-5 isolate (*Enterococcus faecium*) was investigated of quality parameters in fermented milk. Viable counts in milk product were higher than 10^6 CFU/mL until the 28th day of storage (Table 20). For exerting potential benefits to the consumer required by Thai Food and Drug Administration (Thai FDA)[110].

The pH value exhibit were increase to 5.35 at 14 day, 5.16 day, and 4.93 at 28 day as show as Table 21. The pH drop within the 7 day of storage, as the bacterial produced higher amount of lactic acid through lactose hydrolysis was especially fast during the initial period. The pH value exhibit changes in pH value were not clear ternd

of pH value at storage period due to condition of storage may not be stable. But the commercial fermented milk product were decrease pH value at Storage period[111].

The apparent viscosity of milk product sample with F23-5 isolate as the storage period having higher apparent viscosity. Apparent viscosity were 1.23 Pa.s at 1day, 1.32 Pa.s at 7 day, 1.35 Pa.s at 14day, 1.32 Pa.s at 21 day and 1.27 Pa.s at 28 day respectively. Rheological of fermented milk product sample with F23-5 as the storage period having higher G'(Pa). G' were 137.42 Pa at 1 day, 152.08 Pa at 7 day, 172.22 Pa at 14 day, 179.66 Pa at 12 day and 169.23 Pa at 28 day respectively.

Texture characteristics exhibit Firmness of fermented milk sample perennially increase during storage period and syneresis characteristics in milk product exhibit 0% at 1-21 day. The fermented milk by F23-5 isolate had the highest content of 10% milk powder cause increase highest firmness and lower of percent syneresis. Milk powder was increases the volume of milk solid, the resulted fermented milk product is thicker.

Conclusion

The investigation of seventy five lactic acid bacteria isolated from traditional Thai fermented food product collected in Thailand were screened for bile salt hydrolase activity. Two lactic acid bacteria are F1-1 and F23-5 isolates exhibit higher bile salt hydrolase activity on plate assay. The characterization and identification of probiotic lactic acid bacteria, the stains F1-1 and F23-5 isolates were ability survival ability at pH 3.0 and pH 4.0 as compared to MRS control at pH 6.5. F1-1 and F23-5 isolates exhibit survival in bile percentage at 0.3 % bile and 0.8 % bile. On the basic of the phenotypic characteristic and 16s DNA sequence analyses, F1-1 isolate showed 99.88% similarity to *Lactobacillus pentosus* DSM 20314^T and F23-3 isolate showed 99.93% similarity to *Enterococcus faecium* CGMCC 1.2136^T.

F1-1 and F23-5 isolates were selected to determine their serum lipid profile included Total cholesterol (TC), High-density lipoprotein cholesterol (HDL-C). Low-density lipoprotein cholesterol (LDL-C) and Triglycerides (TG) in rat fed high-fat. Rat fed high-fat with F23-5 isolate group exhibited total cholesterol level 48.2 mg/dl, the

significant lower total cholesterol level in blood sample compare with rat fed high-fat group was 92 mg/dl . Intestinal microbiota analysis exhibit pre-treatment and after-treatement were were decrese bacterial type in the intestinal.

F23-5 isolate was able to decrease total cholesterol level in rat fed high fat, this isolate was selected to study fermentation in milk product. This milk product by F23-5 (*Enterococcus faecium*) was investigate of quality parameters in fermented milk include viable counts, pH value, viscosity characteristics, rheological characteristics, texture characteristics and synersis characteristics. This milk product by F23-5 isolate exhibit good quality parameters in fermented milk was similar to commercial fermented milk product.

The results from this study of probiotic lactic acid bacteria in fermented food product collected in Thailand which are the good source of lactic acid bacteria. F1-1 and F23-5 isolates exhibit higher bile salt hydrolase activity, all isolate can be considered as the good probiotic propertise use to rat fed high-fat diet. According to the results of rat fed high-fat diet and rat fed high-fat diet + probiotic, F23-5 exhibited the significant lower total cholesterol level in blood sample and higher High-density lipoprotein cholesterol compare with control group. Investigation of quality parameters in fermented milk, This milk product by F23-5 isolate exhibit good quality parameters in fermented milk was similar to commercial fermented milk product.

s1. Fijan S. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health. 2014;11(5):4745-67.

2. FAO/WHO. Working group report on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada. 2002.

3. Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N. Health Benefits of Probiotics: A Review. ISRN Nutr. 2013.

4. Behnsen J, Deriu E, Sassone-Corsi M, Raffatellu M. Probiotics: properties, examples, and specific applications. CSH PERSPECT MED. 2013;3(3):74 - 5.

5. Zheng Y, Lu Y, Wang J, Yang L, Pan C, Huang Y. Probiotic properties of Lactobacillus strains isolated from Tibetan kefir grains. PloS one. 2013;8(7):e69868-e9.

6. Helander IM, von Wright A, Mattila-Sandholm TM. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends Food Sci Technol. 1997;8(5):146-50.

7. Naidu AS, Bidlack WR, Clemens RA. Probiotic Spectra of Lactic Acid Bacteria (LAB). Crit Rev Food Sci Nutr. 1999;39(1):13-126.

8. König H, Unden G, Froehlich J. Biology of Microorganisms on Grapes, in Must and in Wine2017.

9. Li Y, X L, Chen X, Jiang M, M D. Lactic acid bacteria as functional starter cultures for the food fermentation industry. China Dairy Industry. 2006.

10. Giraffa G, Chanishvili N, Widyastuti Y. Importance of lactobacilli in food and feed biotechnology. Research in Microbiology. 2010;161(6):480-7.

11. Saarela M, Mogensen G, Fondén R, Mättö J, Mattila-Sandholm T. Probiotic bacteria: safety, functional and technological properties. J Biotechnol. 2000;84(3):197-215.

12. Vinderola CG, Reinheimer JA. Lactic acid starter and probiotic bacteria: a comparative “in vitro” study of probiotic characteristics and biological barrier resistance. Food Res Int. 2003;36(9):895-904.

13. Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, et al. Lipoprotein Management in Patients With Cardiometabolic Risk. Diabetes Care. 2008;31(4):811.

14. Nauta MJ, Andersen R, Pilegaard K, Pires SM, Ravn-Haren G, Tetens I, et al. Meeting the challenges in the development of risk-benefit assessment of foods. Trends Food Sci Technol. 2018;76:90-100.

15. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: A comparison of c-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA. 2001;285(19):2481-5.

16. Ishimwe N, Daliri E, Lee B, Fang F, Du G. The perspective on cholesterol lowering mechanisms of probiotics.2015.

17. Chiang JYL. Bile acids: regulation of synthesis. Journal of lipid research. 2009;50(10):1955-66.

18. Monte MJ, Marin JJG, Antelo A, Vazquez-Tato J. Bile acids: chemistry, physiology, and pathophysiology. World J Gastroenterol. 2009;15(7):804-16.

19. Handali S. Absorption-Enhancing Effects of Bile Salts.2015.

20. Jones BV, Begley M, Hill C, Gahan CGM, Marchesi JR. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. Proc Natl Acad Sci U S A. 2008;105:13580-5.

21. Smet ID, Boever PD, Verstraete W. Cholesterol lowering in pigs through enhanced bacterial bile salt hydrolase activity. Br J Nutr. 2007;79(2):185-94.

22. Begley M, Hill C, Gahan CGM. Bile Salt Hydrolase Activity in Probiotics. Appl Environ Microbiol. 2006;72(3):1729.

23. Ooi L-G, Liong M-T. Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. Int J Mol Sci. 2010;11(6):2499-522.

24. Salminen S, Ouwehand A, Benno Y, Lee YK. Probiotics: how should they be defined? Trends Food Sci Technol. 1999;10(3):107-10.

25. Vijayakumar M, Ilavenil S, Kim DH, Arasu MV, Priya K, Choi KC. In-vitro assessment of the probiotic potential of Lactobacillus plantarum KCC-24 isolated from Italian rye-grass (Lolium multiflorum) forage. Anaerobe. 2015;32:90-7.

26. Govender M, Choonara YE, Kumar P, du Toit LC, van Vuuren S, Pillay V. A review of the advancements in probiotic delivery: Conventional vs. non-conventional formulations for intestinal flora supplementation. AAPS PharmSciTech. 2013;15(1):29-43.

27. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506.

28. Huys G, Botteldoorn N, Delvigne F, De Vuyst L, Heyndrickx M, Pot B, et al. Microbial characterization of probiotics--advisory report of the Working Group "8651 Probiotics" of the Belgian Superior Health Council (SHC). Mol Nutr Food Res. 2013;57(8):1479-504.

29. Sanders TA. Food production and food safety. BMJ (Clinical research ed). 1999;318(7199):1689-93.

30. Florou-Paneri P, Christaki E, Bonos E. Lactic Acid Bacteria as Source of Functional Ingredients. Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes2013.

31. Leroy F, De Vuyst L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol. 2004;15(2):67-78.

32. Soro-Yao AA, Brou K, Amani G, Thonart P, Djè KM. The Use of Lactic Acid Bacteria Starter Cultures during the Processing of Fermented Cereal-based Foods in West Africa: A Review. Tropical life sciences research. 2014;25(2):81-100.

33. Stiles ME, Holzapfel WH. Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol. 1997;36(1):1-29.

34. Kántor A, Kačániová M, Petrová J, Medo J, Hleba L, Rovná K. Identification of lactic acid bacteria isolated from red wine samples by RT-qPCR. JMBFS. 2014;3(Special Issue 1):235-7.

35. Fitzsimons NA, Cogan TM, Condon S, Beresford T. Phenotypic and genotypic characterization of non-starter lactic acid bacteria in mature cheddar cheese. Appl Environ Microbiol. 1999;65(8):3418-26.

36. Terzic-Vidojevic A, Vukasinovic M, Tolinacki M, Nikolic M, Lozo J, Begovic J, et al. Phenotypic and genotypic characterization of non-starter lactic acid bacteria from homemade Azerbaijani dairy products2009. 2576-88 p.

37. Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Meier-Kolthoff JP, et al. Taxonomy, Physiology, and Natural Products of Actinobacteria. Microbiol Mol Biol Rev. 2015;80(1):1-43.

38. Gänzle MG, Vermeulen N, Vogel RF. Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. Food Microbiology. 2007;24(2):128-38.

39. Liu SQ. Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations. Int J Food Microbiol. 2003;83(2):115-31.

40. Ghaffar T, Irshad M, Anwar Z, Aqil T, Zulifqar Z, Tariq A, et al. Recent trends in lactic acid biotechnology: A brief review on production to purification. J RADIAT RES. 2014;7(2):222-9.

41. Shah NP. Functional cultures and health benefits. Int Dairy J. 2007;17(11):1262-77.

42. Gill HS, Guarner F. Probiotics and human health: a clinical perspective. Postgrad Med J. 2004;80(947):516-26.

43. Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S, et al. In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. Am J Clin Nutr. 2001;73(2):386s-92s.

44. Vinderola CG, Costa GA, Regenhardt S, Reinheimer JA. Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. Int Dairy J. 2002;12(7):579-89.

45. Leroy F, De Vuyst L. Functional lactic acid bacteria starter cultures for the food fermentation industry2004. 67-78 p.

46. Viana de Souza J, Silva Dias F. Protective, technological, and functional properties of select autochthonous lactic acid bacteria from goat dairy products. Curr Opin Food Sci. 2017;13:1-9.

47. Shiby VK, Mishra HN. Fermented Milks and Milk Products as Functional Foods—A Review. Crit Rev Food Sci Nutr. 2013;53(5):482-96.

48. Virtanen T, Pihlanto A, Mäkinen S, Korhonen H. Development of antioxidant activity in milk whey during fermentation with lactic acid bacteria2007. 106-15 p.

49. Yerlikaya O. Starter cultures used in probiotic dairy product preparation and popular probiotic dairy drinks. Food Sci Technol. 2014;34(2):221-9.

50. Azhari A. Beneficial Role of Lactic Acid Bacteria in Food Preservation and Human Health: A Review2010. 1213-21 p.

51. Scholz-Ahrens KE, Adolphi B, Rochat F, Barclay DV, de Vrese M, Açil Y, et al. Effects of probiotics, prebiotics, and synbiotics on mineral metabolism in ovariectomized rats — impact of bacterial mass, intestinal absorptive area and reduction of bone turn-over. NFS Journal. 2016;3:41-50.

52. Laulund S, Wind A, Derkx MP, Zuliani V. Regulatory and Safety Requirements for Food Cultures. Microorganisms. 2017;5(2).

53. Preidis GA, Hill C, Guerrant RL, Ramakrishna BS, Tannock GW, Versalovic J. Probiotics, Enteric and Diarrheal Diseases, and Global Health. Gastroenterology. 2011;140(1):8-14.e9.

54. Bai AP, Ouyang Q. Probiotics and inflammatory bowel diseases. Postgrad Med J. 2006;82(968):376.

55. Chassany O, Matuchansky C. Probiotics and the irritable bowel syndrome2008. 616-7; author reply 7 p.

56. so S, Wan MLY, El-Nezami H. Probiotics-mediated suppression of cancer2016. 1 p.

57. Kahouli I, Tomaro-Duchesneau C, Prakash S. Probiotics in colorectal cancer (CRC) with emphasis on mechanisms of action and current perspectives. J Med Microbiol. 2013;62(8):1107-23.

58. de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J. Probiotics—compensation for lactase insufficiency. Am J Clin Nutr. 2001;73(2):421s-9s.

59. Kumar M, Nagpal R, Kumar R. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases2012. 902-17 p.

60. Wurong D, Shi C, Chen M, Zhou J, Long R, Guo X. Screening for lactic acid bacteria in traditional fermented Tibetan yak milk and evaluating their probiotic and cholesterol-lowering potentials in rats fed a high-cholesterol diet2017. 324-32 p.

61. Ohvo-Rekilä H, Ramstedt B, Leppimäki P, Peter Slotte J. Cholesterol interactions with phospholipids in membranes. Prog Lipid Res. 2002;41(1):66-97.

62. Cox R, R. García-Palmieri M. Cholesterol, Triglycerides, and Associated Lipoproteins. 1990.

63. Lecerf J-M, de Lorgeril M. Dietary cholesterol: from physiology to cardiovascular risk. Br J Nutr. 2011;106(1):6-14.

64. Rodwell VW, Bender DA, Botham KM, Kennelly PJ, Weil PA. Harper's illustrated biochemistry2015.

65. Murray RK. Harper's illustrated biochemistry. New York: McGraw-Hill Medical; 2012.

66. Dowhan W, Bogdanov M, Mileykovskaya E. CHAPTER 1 - Functional roles of lipids in membranes. In: Vance DE, Vance JE, editors. Biochemistry of Lipids, Lipoproteins and Membranes (Fifth Edition). San Diego: Elsevier; 2008. p. 1-37.

67. K Spady D, Woollett LA, M Dietschy J. Regulation of Plasma LDL-Cholesterol Levels by Dietary Cholesterol and Fatty Acids1993. 355-81 p.

68. Constantinou C, Karavia E, Xepapadaki E, Petropoulou P-I, Papakosta E, Karavyraki M, et al. Advances in High Density Lipoprotein Physiology: surprises, overturns and promises2015. ajpendo.00429.2015 p.

69. Ockner RK, Manning JA. Fatty acid binding protein. Role in esterification of absorbed long chain fatty acid in rat intestine. J Clin Invest. 1976;58(3):632-41.

70. Fernandez M. Dietary cholesterol provided by eggs and plasma lipoproteins in healthy populations2006. 8-12 p.

71. Schonfeld G, Patsch W, Rudel LL, Nelson C, Epstein M, Olson RE. Effects of dietary cholesterol and fatty acids on plasma lipoproteins. J Clin Invest. 1982;69(5):1072-80.

72. Ehrenstein M. Cholesterol. Chemistry, Biochemistry, and Pathology. Journal of the American Chemical Society. 1959;81(15):4120-1.

73. Lee B. Fundamentals of Food Biotechnology: Second Edition2015. 1-518 p.

74. Jones M, Tomaro-Duchesneau C, Martoni C, Prakash S. Cholesterol lowering with bile salt hydrolase-active probiotic bacteria, mechanism of action, clinical evidence, and future direction for heart health applications2013.

75. Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, Kumar A, et al. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp Diabetes Res. 2012;2012:902917-.

76. Chiang JYL. Bile acid metabolism and signaling. Compr Physiol. 2013;3(3):1191-212.

77. Chiang JYL. Bile acids: regulation of synthesis. J Lipid Res. 2009;50(10):1955-66.

78. Lindquist S, Hernell O. Lipid digestion and absorption in early life: An update2010. 314-20 p.

79. Rani RP, Anandharaj M, Ravindran AD. Characterization of Bile Salt Hydrolase from Lactobacillus gasseri FR4 and Demonstration of Its Substrate Specificity and Inhibitory Mechanism Using Molecular Docking Analysis. Front Microbiol. 2017;8:1004-.

80. Tsai C-C, Lin P-P, Hsieh Y-M, Zhang Z-y, Wu H-C, Huang C-C. Cholesterol-lowering potentials of lactic acid bacteria based on bile-salt hydrolase activity and effect of potent strains on cholesterol metabolism in vitro and in vivo. TheScientificWorldJournal. 2014;2014:690752-.

81. Kim S-J, Park SH, Sin H-S, Jang S-H, Lee S-W, Kim S-Y, et al. Hypocholesterolemic Effects of Probiotic Mixture on Diet-Induced Hypercholesterolemic Rats. Nutrients. 2017;9(3):293.

82. Soltan Dallal MM, Zamaniahari S, Davoodabadi A, Hosseini M, Rajabi Z. Identification and characterization of probiotic lactic acid bacteria isolated from traditional persian pickled vegetables. Am J Infect Control. 2017;12:Doc15-Doc.

83. Mamhoud A, Nionelli L, Bouzaine T, Hamdi M, Gobbetti M, Rizzello CG. Selection of lactic acid bacteria isolated from Tunisian cereals and exploitation of the use as starters for sourdough fermentation. Int J Food Microbiol. 2016;225:9-19.

84. Brashears M, Jaroni D, Trimble J. Isolation, Selection, and Characterization of Lactic Acid Bacteria for a Competitive Exclusion Product To Reduce Shedding of Escherichia coli O157:H7 in Cattle2003. 355-63 p.

85. Feng Guo C, Wei Zhang L, Yan Li J, Chun Zhang Y, Hui Xue C, Xi Yi H, et al. Screening of Bile Salt Hydrolase-Active Lactic Acid Bacteria for Potential Cholesterol-Lowering Probiotic Use2011. 139-46 p.

86. Tsai C-C, Lin P-P, Hsieh Y-M, Zhang Z-Y, Wu H-C, Huang C-C. Cholesterol-Lowering Potentials of Lactic Acid Bacteria Based on Bile-Salt Hydrolase Activity and Effect of Potent Strains on Cholesterol Metabolism In Vitro and In Vivo2014. 690752 p.

87. Wang SC, Chang CK, Chan SC, Shieh JS, Chiu CK, Duh P-D. Effects of lactic acid bacteria isolated from fermented mustard on lowering cholesterol. Asian Pac J Trop Biomed. 2014;4(7):523-8.

88. Tanasupawat S, Pakdeeto A, Thawai C, Yukphan P, Okada S. Identification of lactic acid bacteria from fermented tea leaves (miang) in Thailand and proposals of Lactobacillus thailandensis sp. nov., Lactobacillus camelliae sp. nov., and Pediococcus siamensis sp. nov2007. 7-15 p.

89. Bordoni A, Amaretti A, Leonardi A, Boschetti E, Danesi F, Matteuzzi D, et al. Cholesterol-lowering probiotics: in vitro selection and in vivo testing of bifidobacteria. Appl Microbiol Biotechnol. 2013;97(18):8273-81.

90. Salaj R, Stofilová J, Soltesová A, Hertelyová Z, Hijová E, Bertková I, et al. The effects of two Lactobacillus plantarum strains on rat lipid metabolism receiving a high fat diet. TheScientificWorldJournal. 2013;2013:135142-.

91. Lecomte V, Kaakoush NO, Maloney CA, Raipuria M, Huinao KD, Mitchell HM, et al. Changes in gut microbiota in rats fed a high fat diet correlate with obesity-associated metabolic parameters. PloS one. 2015;10(5):e0126931-e.

92. Wang Y, Xu N, Xi A, Ahmed Z, Zhang B, Bai X. Effects of Lactobacillus plantarum MA2 isolated from Tibet kefir on lipid metabolism and intestinal

microflora of rats fed on high-cholesterol diet. Appl Microbiol Biotechnol. 2009;84(2):341-7.

93. Senaka Ranadheera C, Evans CA, Adams MC, Baines SK. Probiotic viability and physico-chemical and sensory properties of plain and stirred fruit yogurts made from goat's milk. Food Chemistry. 2012;135(3):1411-8.

94. Krasaekoopt W, Bhandari B, Deeth H. The inuence of coating materials on some properties of alginate beads and survivability of microencapsulated probiotic bacteria2004. 737-43 p.

95. Vivar-Quintana AM, Beneitez De La Mano E, Revilla I. Relationship between somatic cell counts and the properties of yoghurt made from ewes' milk. Int Dairy J. 2006;16(3):262-7.

96. Ramírez-Santiago C, Lobato-Calleros C, Espinosa-Andrews H, Vernon-Carter EJ. Viscoelastic properties and overall sensory acceptability of reduced-fat Petit-Suisse cheese made by replacing milk fat with complex coacervate2012. 383-98 p.

97. Lee WJ, Lucey JA. Structure and Physical Properties of Yogurt Gels: Effect of Inoculation Rate and Incubation Temperature. J Dairy Sci. 2004;87(10):3153-64.

98. Begley M, Hill C, Gahan CGM. Bile Salt Hydrolase Activity in Probiotics. Applied and Environmental Microbiology. 2006;72(3):1729.

99. Wang Y, Xu N, Xi A, Ahmed Z, Zhang B, Bai X. Effects of Lactobacillus plantarum MA2 isolated from Tibet kefir on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. Applied Microbiology and Biotechnology. 2009;84(2):341-7.

100. Shehata M, El-Sohaimy S, el-sahn M, Youssef M. Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity2016.

101. Villena J, Kitazawa H. Probiotic Microorganisms: A Closer Look. Microorganisms. 2017;5(2):17.

102. Nami Y, Haghshenas B, Vaseghi Bakhshayesh R, Mohammadzadeh Jalaly H, Lotfi H, Eslami S, et al. Novel autochthonous lactobacilli with probiotic aptitudes as a main starter culture for probiotic fermented milk2018.

103. Hassanzadazar H, Ehsani A, Mardani K, Hesari J. Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. VRF Journal 2012;3(3):181-5.

104. Wang S, Yuan X, Dong Z, Li J, Guo G, Bai Y, et al. Characteristics of isolated lactic acid bacteria and their effects on the silage quality. J Anim Sci. 2017;30(6):819-27.

105. Velrajan M. Preliminary Study To Enhance The Growth Rate Of Bacteria2016.

106. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Primary care. 2013;40(1):195-211.

107. Tok E, Aslim B. Cholesterol removal by some lactic acid bacteria that can be used as probiotic2010. 257-64 p.

108. Zhang F, Qiu L, Xu X, Liu Z, Zhan H, Tao X, et al. Beneficial effects of probiotic cholesterol-lowering strain of Enterococcus faecium WEFA23 from infants on diet-induced metabolic syndrome in rats2016.

109. Peng Y, Wu C, Yang J, Li X. Gut microbial diversity in rat model induced by rhubarb. Experimental animals. 2014;63(4):415-22.

110. Velasco RP, Chaikledkaew U, Myint CY, Khampang R, Tantivess S, Teerawattananon Y. Advanced health biotechnologies in Thailand: redefining policy directions. J Transl Med. 2013;11:1-.

111. Arioui F, Ait Saada D, Cheriguene A. Physicochemical and sensory quality of yogurt incorporated with pectin from peel of Citrus sinensis. Food Sci Nutr 2016;5(2):358-64.

REFERENCES

VITA

NAME	preeyarach whisetkhan
DATE OF BIRTH	19 July 1992
PLACE OF BIRTH	kalasin,Thailand
INSTITUTIONS ATTENDED	faculty of medicine
HOME ADDRESS	162/2 moo2 Khaowong Kalasin 14606
PUBLICATION	1. Whisetkhan p. Taweechotipart M. and Pachekrepapol U. Isolation and identification of probiotic lactic acid bacteria with cholesterol lowering potential. International Scientific Conference on Probiotics, Prebiotics, Gut (IPC), june, 18-21, 2018, Hungary, Budapest. 2. Whisetkhan P. Pachekrepapol U. Taweechotipatr M. isolation and identification of probiotic acid bacteria for bile salt hydrolase activity. The 11th Srinakarinwirot University Research Conference 20-21, 2018, Bangkok, Thailand. 3. Ladda B. Whisetkhan P. Tanasupawat S. Taweechotipatr M. Probiotic Properties of lactic acid bacteria isolated from human origin. 6-8, July, 2017, Korea.