การตรวจหาแลคโตบาซิลลัสในกระเพาะอาหารของผู้ป่วยที่มีอาการปวดท้องบริเวณท้องส่วนบน และบทบาทในการลดการสร้าง Tumor Necrosis Factor-α ในหลอดทดลอง

นางสาววิมลรัตน์ ปานเพ็ชร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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DETECTION OF *LACTOBACILLUS* IN THE STOMACH OF DYSPEPTIC PATIENT AND ITS ROLE IN THE SUPPRESSION OF TUMOR NECROSIS FACTOR-α PRODUCTION *IN VITRO*

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วิมลรัตน์ ปานเพ็ชร : การตรวจหาแลคโตบาซิลลัสในกระเพาะอาหารของผู้ป่วยที่มีอาการปวดท้องบริเวณท้องส่วนบนและบทบาทในการลดการสร้าง Tumor Necrosis Factor-α ในหลอดทดลอง (DETECTION OF *LACTOBACILLUS* IN THE STOMACH OF DYSPEPTIC PATIENTS AND ITS ROLE IN THE SUPPRESSION OF TUMOR NECROSIS FACTOR-α PRODUCTION *IN VITRO*) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. สมหญิง ธัมวาสร, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. พญ. ควงพร ทองงาม, 236 หน้า

แลคโตบาซิลลัสเป็นจุลชีพที่พบเป็นประจำในระบบทางเดินอาหารของสัตว์เลี้ยงลูกด้วยนมและบางสายพันธุ์สามารถกดการสร้าง proinflammatory cytokines หลายชนิดรวมทั้ง tumor necrosis factor-α (TNF-α) ในการศึกษาครั้งนี้ได้เพาะแยกและวิเคราะห์สปีชีส์ของเชื้อแลคโตบาซิลลัสจากชิ้นเนื้อกระเพาะอาหารและคอของผู้ป่วยที่มีอาการปวดท้องบริเวณท้องส่วนบน จำนวน 272 ราย ซึ่งแยกเป็นกลุ่มตามผลการตังเกตจากการส่องกล้องออกเป็น 3 กลุ่ม คือ กลุ่มที่หนึ่ง 70 ราย เป็นผู้ป่วยที่มีกระเพาะอาหารอักเสบเล็กน้อย กลุ่มที่สอง 158 ราย เป็นผู้ป่วยที่มีกระเพาะอาหารอักเสบอย่างรุนแรง และกลุ่มที่สาม 44 ราย เป็นผู้ป่วยที่มีแผลในกระเพาะอาหาร

ผลการเพาะแยกเชื้อพบแลคโตบาซิลลัสในกระเพาะอาหารผู้ป่วยจำนวน 57 ราย (20.96%) โดยแยกได้จากกลุ่มที่หนึ่ง 9 ราย (12.85%) กลุ่มที่สอง 32 ราย (20.25%) และกลุ่มที่สาม 16 ราย (36.36%) ผลการวิเคราะห์ทางสถิติพบว่า ความชุกของเชื้อในกลุ่มที่สองกับกลุ่มที่สามและกลุ่มที่หนึ่งกับกลุ่มที่สามมีความแตกต่างกันอย่างมีนัยสำคัญ ผลการเพาะแยกเชื้อแลคโตบาซิลลัสจากคอของผู้ป่วย พบเชื้อในผู้ป่วย 103 ราย (37.87%) โดยแยกได้จากกลุ่มที่หนึ่ง 25 ราย (35.71%) กลุ่มที่สอง 57 ราย (36.08%) และกลุ่มที่สาม 21 ราย (47.73%) ผลการวิเคราะห์ทางสถิติพบว่าความชุกของเชื้อในผู้ป่วยแต่ละกลุ่มไม่แตกต่างกันกันอย่างมีนัยสำคัญ

เมื่อนำเชื้อแลคโตบาซิลลัสที่แยกได้จากชิ้นเนื้อกระเพาะอาหารผู้ป่วย 57 ราย มาทดสอบความสามารถในการลดการสร้าง TNF-α โดย THP-1 monocytic cells ที่ถูกกระตุ้นด้วย lipopolysaccharide (LPS) พบว่า ในผู้ป่วย 31 ราย (54.39%) ลดการสร้าง TNF-α อย่างมีนัยสำคัญ ($p<0.05$) โดยแยกได้จากกลุ่มที่หนึ่ง 7 ราย (77.78%) กลุ่มที่สอง 18 ราย (56.25%) และกลุ่มที่สาม 6 ราย (37.5%) ผลการวิเคราะห์ทางสถิติพบว่า ความชุกของเชื้อที่สามารถลดการสร้าง TNF-α ในผู้ป่วยกลุ่มที่หนึ่งกับกลุ่มที่สามแตกต่างกันอย่างมีนัยสำคัญ ($p=0.053$) อย่างไรก็ตามผลการวิเคราะห์ด้วย multivariate analysis พบว่า ความชุกของเชื้อที่ลดการสร้าง TNF-α ในกลุ่มที่หนึ่งกับกลุ่มที่สามไม่มีความแตกต่างกันอย่างมีนัยสำคัญ ($p=0.985$)

เชื้อแลคโตบาซิลลัสที่สามารถลดการสร้าง TNF-α ในการศึกษาครั้งนี้ได้แก่ *Lactobacillus plantarum, L. murinus* ที่แยกได้ทั้งหมด *L. salivarius, L. gasseri* และ *L. casei* group บาง isolate ส่วนเชื้อ *L. fermentum, L. mucosae* และ *L. oris* ที่แยกได้ทั้งหมดไม่สามารถลดการสร้าง TNF-α สปีชีส์ของเชื้อแลคโตบาซิลลัสที่พบมากที่กระเพาะอาหารและคอคือ *L. fermentum* และ *L. salivarius* ผู้ป่วยที่พบแลคโตบาซิลลัสทั้งสองบริเวณมี 38 ราย และมีผู้ป่วย 28 ราย (73.68%) ที่มีเชื้อเหมือนกันอย่างน้อย 1 สปีชีส์ทั้งในกระเพาะอาหารและคอ ผลการศึกษาบ่งชี้ว่าเชื้อแลคโตบาซิลลัสบางสปีชีส์ที่กระเพาะอาหารอาจจะมีจุดเริ่มต้นมาจากคอของผู้ป่วยและเชื้อแลคโตบาซิลลัสที่พบในกระเพาะอาหารอาจเป็นปัจจัยหนึ่งที่มีผลกับพยาธิสภาพและความรุนแรงของโรคแผลกระเพาะอาหาร

สาขาวิชา จุลชีววิทยาทางการแพทย์ ลายมือชื่อนิสิต.....
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KEY WORD : MICROFLORA/ *LACTOBACILLUS* / TUMOR NECROSIS FACTOR-α/ THP-1 CELL / IMMUNOMODULATION

WIMONRAT PANPETCH : DETECTION OF *LACTOBACILLUS* IN THE STOMACH OF DYSPEPTIC PATIENTS AND ITS ROLE IN THE SUPPRESSION OF TUMOR NECROSIS FACTOR-α PRODUCTION *IN VITRO*. THESIS PRINCIPAL ADVISOR : ASSOC. PROF. SOMYING TUMWASORN, Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. DUANGPORN THONGNGAM, M.D., 236 pp.

Lactobacillus species represent indigenous microorganisms of the mammalian gastrointestinal tract and some specific strains can suppress the production of a number of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α). In this study, *Lactobacillus* spp. were isolated from gastric biopsies of 272 dyspeptic patients that were divided into three groups by endoscopic findings as follows: group one, 70 patients with mild gastritis; group two, 158 patients with severe gastritis and group three, 44 patients with peptic ulcer.

Bacterial culture of gastric biopsies yielded 57 patients (20.96%) which were categorized into 9 patients (12.85%) in group one, 32 patients(20.25%) in group two and 16 patients (36.36%) in group three. Statistical analyses revealed that the prevalence of *Lactobacillus* in patients groups one and two were not significantly different ($p>0.05$) but significantly different in patients groups two and three and patients groups one and three ($p<0.05$).

Bacterial culture of throat swabs yielded 103 patients (37.87%) which were categorized into 25 patients (35.71%) in group one, 57 patients (36.08%) in group two and 21 patients (47.73%) in group three. Statistical analyses revealed that the prevalence of *Lactobacillus* in each group of patients were not significantly different ($p>0.05$).

The immunomodulating activities of *Lactobacillus* isolated from gastric biopsies of 57 patients revealed 31 patients (54.39%) significantly suppressed LPS-activated TNF-α production by THP-1 monocytic cells ($p<0.05$). These TNF-α-inhibitory isolates were 7 patients (77.78%) in group one, 18 patients (56.25%) in group two and 6 patients (37.5%) in group 3. Statistical analyses revealed that the prevalence of TNF-α-inhibitory *Lactobacillus* isolates in patients groups one and two v.s. groups two and three were not significantly different ($p>0.05$) but significantly different in patients groups one and three ($p=0.053$). However, multivariate analysis of the prevalence of TNF-α-inhibitory *Lactobacillus* in patients groups 1 and 3 was not significantly different ($p=0.985$)

TNF-α-inhibitory *Lactobacillus* found in this study were all isolates of *Lactobacillus plantarum, L. murinus* and some isolates of *L. salivarius, L.gasseri and L.casei* group. On the contrary, all isolates of *L. fermentum, L. mucosae* and *L. oris* did not suppress TNF-α production. Predominate species found in both gastric biopsies and throat were *L. fermentum* and *L. salivarius.* Of 38 patients from whom *Lactobacillus* spp. were recovered from both gastric biopsies and throat swabs, 28 (73.68%) had at least one isolate of the same species. The results of this study suggested that some *Lactobacillus* species detected in gastric biopsies originate from throats and *Lactobacillus* species in the stomach might be a factor contributing to the pathogenesis of peptic ulcer.

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Principal Advisor's signature Somying Tumwasorn
Co-Advisor's signature Duangporn Thongngam

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LIST OFABBREVIATIONS

A	=	adenosine
bp	=	base pair
CO_2	=	cabondioxide
$^{\circ}C$	=	degree Celsius
CFU	=	colony forming unit
dATP	=	deoxyadenosine 5'-triphosphate
dCTP	=	deoxycytidine 5'-triphosphate
ddATP	=	dideoxyadenosine 5'-triphosphate
ddCTP	=	di deoxycytidine 5'-triphosphate
ddGTP	=	dideoxyguanosine 5'-triphosphate
ddTTP	=	dideoxytymidine 5'-triphosphate
DDW	=	double distilled water
ddNTPs	=	dideoxynucleotide-tri-phosphate
dGTP	=	deoxyguanosine 5'-triphosphate
DI	=	deionised water
DNA	=	deoxynucleotide-tri-phosphate
dNTPs	=	deoxynucleotide-tri-phosphate
dTTP	=	deoxythymidine-5'-triphosphate
DW	=	distilled water
EDTA	=	ethylenediamine tetraaceticacid
et al.	=	et alii
e.g.	=	exempli gratia
g	=	gram
G	=	guanosine
HCl	=	hydrochloric acid
HPLC	=	high performance liquid chromatography
h	=	hour
H_2SO_4	=	sulfuric acid
i.e.	=	id est
KCl	=	potassium chloride
kDa	=	kilometer Daltan
KH_2PO_4	=	potassium phosphate monobasic

M	=	molar
mg	=	milligram
$MgCl_2$	=	magnesium chloride
min	=	minite(s)
ml	=	mililiter
mM	=	milimolar
NaCl	=	sodium chloride
$NaHCO_3$	=	sodium bicarbonate or sodium hydrogen carbonate
Na_2HPO_4	=	sodium phosphate dibasic, anhydrous
NaOH	=	sodium hydroxide
PCR	=	polymerase chain reaction
pmol	=	picomol
p.s.i.	=	pounds/inch ^{2}
RNA	=	ribonucleic acid
rRNA	=	ribosomal ribonucleic acid
16SrRNA	=	sixteen subunit ribosomal ribonucleic acid
23SrRNA	=	twenty three subunit ribosomal ribonucleic acid
sec	=	second
T	=	thymidine
TBE	=	Tris-Boric Acid-EDTA
Taq	=	Themus aquaticus
Tris	=	Tris-(Hydroxymethyl)-aminoethane
U	=	unit
µg	=	microgram
µl	=	microliter
µM	=	micromolar
UV	=	ultraviolet
V	=	volt
WHO	=	World Health Oganization
w/v	=	weight per volume

CHAPTER I

INTRODUCTION

The stomach is digestive tract system was received food from mouth passed to esophagus. The stomach acts essentially as a mixing reservoir for food during acid-pepcin digestion. Hydrochloric acid and pepsin are producted by gastric mucosa (1). The stomach is divided into five anatomic region; the cardia, fundus, body, antrum and pyloric sphincter (2-4). The gastric walls consist of the major layers are mucosa, submucosa, muscularis propria and serosa together with gastric vessels and nerves (5).

Dyspepsia is global problem was recurrent of pain or discomfort located in the upper abdomen which found that approximately 25% of general population (6). Dyspepsia was recurrent of pain or discomfort located in the malignancy, colitis, pancreatic and biliary tract disorder (7). The symptom of dyspepsia was varied various and compared with several symptoms. The causes of dyspepsia were included a number of foods, prolong use nonsteroidal anti-inflammatory drugs and other medications, systemic disorder disease, infected with *Helicobacter pylori* and gastrointestinal tract disease that included peptic ulcer disease, stomach cancer, gastric oresophageal malignancy.

Dyspepsia patients were identified cause by using esophagogastroduodenal endoscopy and pathology of gastric biopsies which found endoscopic normal, mild gastritis, each type of gastritis and peptic ulcer. Gastritis is simply defined as inflammation of the gastric mucosa (4), which endoscopist was refered to abnormalities of stomach and visualized by endoscopic finding: for example, nodular gastritis, hemorrhagic gastritis, diffuse gastritis, granularity and erythema (8). Inflammation of the stomach, is usually considered as acute and chronic gastritis (1). The mechanisms of gastric inflammation are not clearly. However, now suggests that an imbalance of aggressive factor. They defensive factor that are aggressive factors such as acid production or pepsin, bile, *H. pylori* infection and defensive factors such as mucus production, bicarbonate, surface hydrophobicity, cell turn over and blood flow (9). Peptic ulcer disease was defected in the gastrointestinal mucosa which involved of muscularis

mucosa and into the submucosal layer. The causes of gastritis and gastric ulcer are occurred from several causes such as the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and *H. pylori* infection (10-12). In 1983, Barry Marshall and Robin Warren identified *H. pylori* as a bacterium closely associated with chronic gastritis and peptic ulcer (13). Pathophysiology of gastrointestinal tracts disease was associated roles of cytokines released in prolonged used of NSAIDs and *H. pylori* induced gastritis and peptic ulcer that induced interleukin-1β (IL-1β), IL-2, IL-6, IL-8, IL-12 and tumor necrosis factor- α (TNF-α) production (14-16). Previous reported, *H. pylori* is the definitive carcinogen for stomach cancer and is known to induce proinflammatory cytokines, such as TNF-α and IL-1 in the stomach (17). The levels of TNF-α in gastric juice and in gastric biopsy homogenate supernatants in patients with *H. pylori*-positive gastritis were found to be significantly higher than those in person without *H. pylori* infection (15, 18).

Tumor necrosis factor-alpha (TNF-α) is a member of a family of cytokines. TNF-α is a highly pleiotropic cytokine that plays a central role in inflammation (19). TNF-α is a major mediator of inflammation and it has been associated in the pathogenesis of a wide spectrum of human diseases, including sepsis, diabetes, osteoporosis, allograft rejection and autoimmune diseases such as multiple sclerosis (20), rheumatoid arthritis, inflammatory bowel diseases (21, 22) and inflammation of stomach (23). TNF-α is beneficial in activating the innate immune response, inappropriate production of TNF-α leads to inflammation, tissue destruction, and organ injury. Monocytes and macrophages are major cellular components of the innate of the immune system which ability to produce TNF-α, in response to bacteria and bacterial fragments, such as lipopolysaccharide (LPS) (24). TNF-α is a key mediator in a host response to infections. LPS (endotoxin), a constituent of the outer membrane of gram-negative bacteria, can initiate a cascade of inflammatory mediators that can lead to systemic inflammation (25). Previous study have investigated the effects of LPS on the expression of cytokines secreted by bovine polymorphonuclear leukocytes (PMN), they detected the expression of TNF-α by ELISA (26). Lipoteichoic acid (LTA) and lipopolysaccharide (LPS), the toxicants from bacteria, are potent inducers of inflammatory cytokines, such as TNF-α in macrophages notably, increasing evidence suggests that macrophages also play an important role in the development of the low-grade inflammation (27).

The microflora is microorganism which normally inhabit the healthy human body or other natural environment and not disease causing. The commensal bacteria help to defend against colonization by pathogen. The human gastrointestinal is colonized by 10^{13}- 10^{14} bacteria of 400 different species and subspecies (28). The human gastrointestinal tract constitutes a complex community of microflora. The microflora establishes after birth and stable of colonize in human body. The stomach contains microflora about 10^{3}cfu/ml. The lower counts contributed to highly acidic which destroy most oral bacteria. The conditions are highly acidic and also a naerobic-not much diversity. The microflora of the stomach are constitute of gram positive and anaerobic bacteria with *Peptostreptococcus* sp., *Lactobacillus* sp., *Staphylococcus* sp., and *Streptococcus* sp. (28).

The member of genus *Lactobacillus* is gram-positive facultative an aerobic bacteria, which some strains are microaerophilic to a naerobic, non-spore-forming bacteria and non motile but few strains are motile by peritrichous flagella. They are member of the lactic acid bacteria group. There are no production of catalase, oxidase, indole and no reduction of nitrate. The most strain of *Lactobacillus* are vancomycin resistance, some strains are vancomycin susceptible (29). Genus *Lactobacillus* consists of more than 175 recognized species (www. http://www.dsmz.de). Growth is best by anaerobic, facultative anaerobic and microaerobic conditions and require carbon dioxide (CO_2) (29).

Lactobacilli have been detected in diverse environments. They are used in the production of foods prepared by means of lactic acid fermentation such as dairy products, fermented vegetables and fermented meats. They are found in plant material such as in foodstuffs, silage and agriculture products (30). *Lactobacillus* is commonly associated with the body of humans and animals (31, 32). They are microflora in the oral cavity (33), gastrointestinal tracts and vagina (29, 33, 34). *Lactobacillus* some strains can resist from gastric acid and bile salts and adherent to intestinal tissue. *Lactobacillus* has been isolated and identified from gastric mucosa of healthy volunteers (35).

Lactobacillus is probiotics bacteria which probiotics are defined as live microbial feed supplement that beneficially affects the human and animal by improving its intestinal microbial balance. These bacteria must belong to the natural microflora in order to survive the acid environment of digestive tract system, the natural balance of the gut microflora can be restored and the animal returned to its normal nutrition, growth and health status (36-39). *Lactobacillus* has been used as probiotic against gastrointestinal tracts infection (40), against enteric pathogen (41). *Lactobacillus* isolated from intestinal has been repored can inhibit growth of *Clostridium difficile* (42). *Lactobacillus rhamnosus*, strain GG, has shown efficacy in clinical trials for the prevention of antimicrobial-associated diarrhea (43). Probiotics *L. acidophilus* that have demonstrated at least some promise as prophylaxis for diarrhea (43). *L. reuteri* is effective as a therapeutic agent in acute rotavirus diarrhea in children (44-47). Several *Lactobacillus* species have shown clinical efficacy as a treatment for vaginal infections. The study characterized human *Lactobacillus* isolates from their capacity to interfere with the growth of *Candida albicans* identified as *L. fermentum* and designated *L. fermentum* Ess-1 was significantly inhibited the growth of *C. albicans* which vaginal candidiasis pathogens (48). *L. acidophilus* or *L. rhamnosus* GR-1 and *L. fermentum* RC-14 reduced the recurrences of bacterial vaginosis (BV) (49, 50). Putative mechanisms of action of probiotics include production of pathogen-inhibitory substances, inhibition of pathogen attachment, inhibition of the action of microbial toxins, stimulation of immunoglobulin A, and trophic effects on intestinal mucosa (43). The intestinal microflora has been attributed many beneficial properties that increased maturation of the gut (51) pathogen antagonism (52-54), and immune modulation (55, 56)

Lactobacillus species have been suggested that the potential to ameliorate or prevent a variety of diseases through modulation of the host's immune system, specifically cellular immune responses (57). *L. casei* strain Shirota (LcS) has been shown to induce IFN-gamma, IL-1β and TNF-α production, in the thoracic cavity of mice, which inhibition of tumour growth and increased survival (57). Lactobacilli have been reported to effectively inhibit TNF-α production *in vitro* (32, 58). *Lactobacillus* conditioned media *L. rhamnosus* GG can inhibit TNF-α by LPS-activated macrophage (58). Similarly, *L. rhamnosus* GG-conditioned media decreases TNF-α production of *Helicobacter*-activated peritoneal macrophages (32). Previous study, in animal model

which *H. hepaticus*-challenged IL-10-deficient murine colitis model, lactobacilli showed the effects by modulation of mucosal inflammatory responses (59). *L. reuteri* and *L. pacracesei* isolated from without colitis mice are demonstrated that TNF-α inhibition properties on LPS-activated macrophages (32). Inflammatory bowel disease (IBD) refers to disorders of unknown cause that are characterized by chronic or recurrent intestinal inflammation, such as ulcerative colitis, Crohn's disease, and pouchitis (60). Several studies showed interesting effects of probiotics on inflammatory bowel disease in animals. Reported, exogenous administration of *L. reuteri* R2LC reducted the development of acetic acid-induced colitis in the rat (61). Colonization of *H. pylori* in the gastric mucosa is strongly associated with gastritis, duodenal and gastric ulcers, and stomach malignancies (60). Antagonistic actions of some *Lactobacillus* strains against *H. pylori* in vitro were reported (62). Previous study reported, *L. salivarius* was efficiently eradicated *H. pylori* and reduced the inflammation in *H. pylori* infected in gnotobiotic murine model which using by oral administration (63). The yogurt containing of *L. gasseri* OLL2716 improve *H. pylori* infection-induced gastric mucosal imflammation. The investigated of *L. gasseri* OLL2716 (LG21) exibites a gastroprotective action against of acute gastric lesion or antral ulcer in rats in dose-dependently (64). Human trials have been reported yogurt containing *L. gasseri* OLL2716 (LG21) suppressing *H. pylori* colonization and reducing gastric mucosal inflammation in humans (65, 66). IL-8 is chemokine which a potent neutrophil chemoattractant and activating agent, accumulating evidence indicates that IL-8 plays a major role in the mucosal inflammation caused by *H. pylori* infection (67, 68). *L. gasseri* OLL2716 (LG21) suppress *H.pylori*-induce IL-8 production in human gastric epithelial cell line (MKN45). TNF-α is well-known to induce IL-8 production in gastric epithelial cells. Indeed, in the present study 10^8 CFU/mL LG21 inhibited the TNF-α-stimulated IL-8 production. Furthermore they study LG21 can inhibit the adhesion of *H. pylori* to host cells which associated with dose dependent of LG21. Finally, they found that live LG21 can suppress *H. pylori*-induced IL-8 production in gastric mucosa of patients (66).

The human stomach is an inhospitable environment for microorganisms because of acidic conditions and other antimicrobial factors. The population of bacteria in stomach about 10^3 CFU/ml (69) which is few bacterial species can resist to the hydrochloric acid in saliva and gastric acid-tolerant (70). The reported *L. acidophilus* has an inhibitory effect on *H. pylori* isolated from peptic ulcer patients and could enhance

antibiotic therapy for *H. pylori* eradication (71, 72). Previous study *Lactobacillus* was considered to be indigenous of stomach of rat, mice and pig (73). *Lactobacillus* is colonized on surface of the squamous keratinized epithelium cell in part of stomach. Previous study *Lactobacillus* can isolate from biopsy of stomach of conventional rats (74).

Lactobacilli are considered to have benefitial effects for health of human and animal. They are interference to infection of pathogens in gastrointestinal tracts including stomach and intestine. *Lactobacillus* has been ability for regulation of host immume system. Interestingly, immunomodulation properties of lactobacilli in gastrointestinal tracts are demonstrated. Consequently, the effects that are suggested for diverse *Lactobacillus* probiotic include prophylaxis and treatment of gastrointestinal infections, which includes traveler's diarrhea, Inflammatory bowel disease (IBD) and ability to modulate inflammatory responses in gastrointestinal tracts such as gastritis, gastric ulcer, duodenum ulcer and stomach cancer.

This study was focused on the detection of *Lactobacillus* in throat and gastric tissue and tested the TNF-α suppressing ability of *Lactobacillus* that isolated from stomach of dyspeptic patients which categorized into three groups; mild gastritis, severe gastritis and peptic ulcer. The role of *Lactobacillus*-mediated immunomodulation in the severity of these diseases was investigated.

CHAPTER II

OBJECTIVE

THE OBJECTIVES OF THIS STYDY WERE

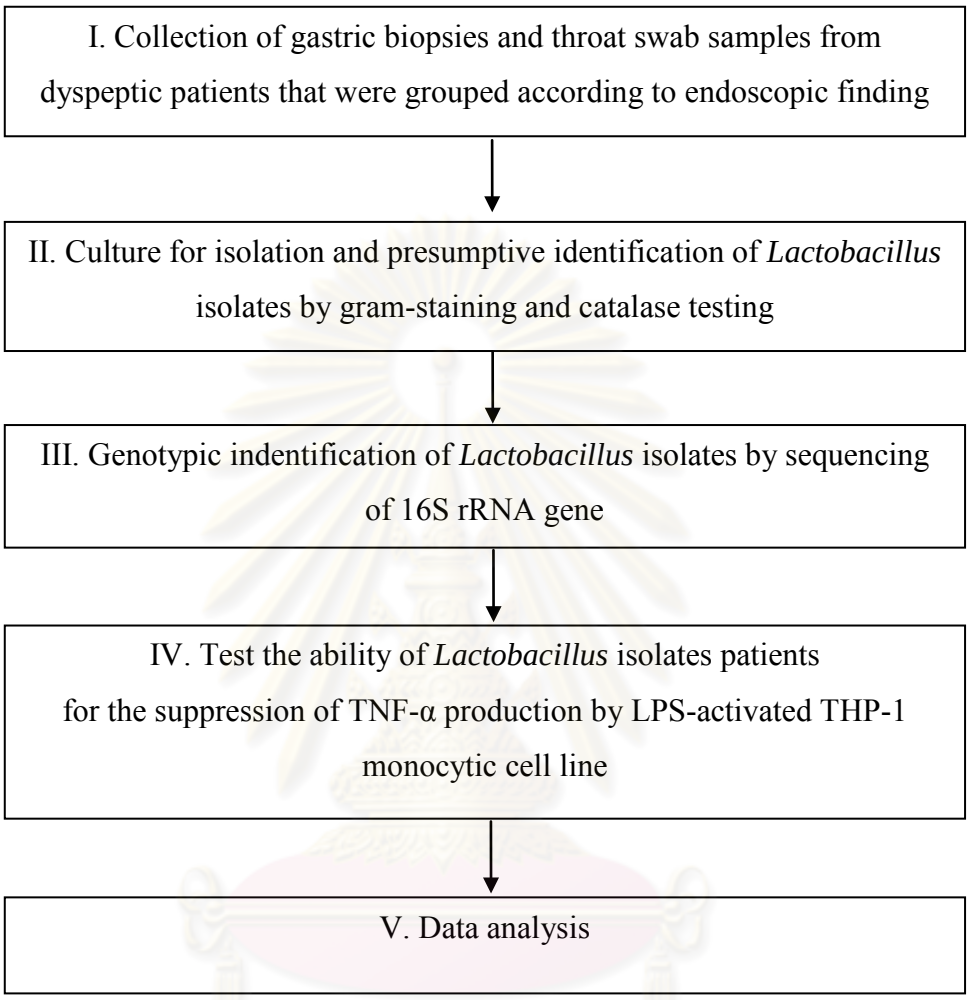
1. Isolate and identify *Lactobacillus* from gastric biopsies and throat swabs of dyspeptic patients with mild gastritis, severe gastritis and peptic ulcer

2. Test the immunomodulation properties of *Lactobacillus* isolated from gastric biopsies of dyspeptic patients for the suppression of tumor necrosis factor-alpha (TNF-α) by lipopolysaccharide (LPS)-activated THP-1 monocytic cells

3. Compare the prevalence and number of TNF-α inhibitory *Lactobacillus* isolates in each group of patients

4. Compare species of *Lactobacillus* isolates from gastric biopsy and throat swab of each patients

CONCEPTUAL FRAMEWORK



CHAPTER III

LITERATURE REVIEW

STOMACH

The stomach is a J-shaped tube, which between of esophagus and duodenum and in the upper left portion of the abdomen. The stomach is the part of digestive tract system which receives food from the esophagus, mixes with gastric juice, initiates the digestion of protein and absorption and carried food into the small intestine (5). The lesser and greater curvatures are on the right and left side of stomach, respectively. The stomach is divided into four part; the cardia, fundus, body, and pyloric regions or antrum (2, 3).

The cardia is the most proximal part of the stomach. The cardia is the small area near the cardiac sphincter which contained approximal 0.5-2.0 cm. of the stomach. The fundus is the balloons superior of the stomach that lies above an unreal horizontal plane that allow through the esophagogastric junction. The body is a large main part of the stomach which located between the fundus and pyloric region (antrum). The rugal folds of the fundus and body (synonyme = corpus) give way to the smooth mucosa of the antrum. The pyloric region or antrum is the smaller distal one fourth to on third of the stomach (10, 11). The antrum is a funnel-shaped portion that narrows and become the pyloric canal that connects the stomach with the duodenum as shown in Figure 1 (2, 3, 5).

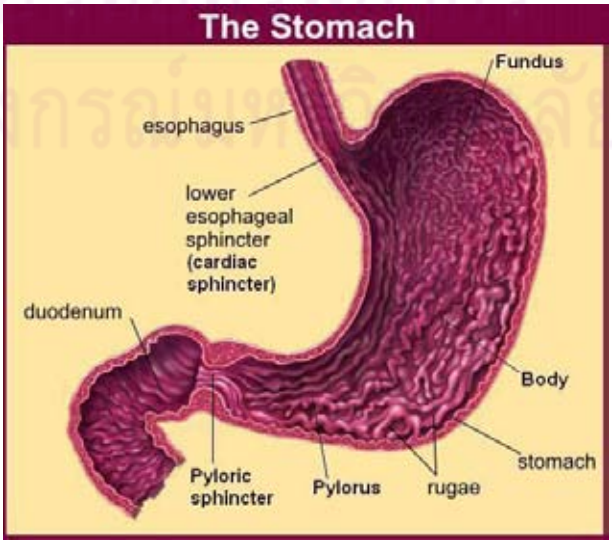


Figure 1. Anatomic divisions of the stomach

MICROSCOPIC ANATOMY OF STOMACH

The gastric walls consist of the major layers are mucosa, submucosa, muscularis propria and serosa together with gastric vessels and nerves (5).

Mucosa is the thick layer with a soft and smooth surface. In the stomach mucosa are folded into numerous folds or rugae. The mucosa consists of an epithelium cover the surface and lines the pits and grands, surrouding connective tissue called the lamina propria, and the muscularis mucosae. The muscularis mucosa is a thin layer of smooth muscle fibers lying external to the layer of grand which forms the inferior margin of the mucosa and separates of mucosa from submucosa (3, 5, 10, 75).

Submucosa is the layer under the mucosa which is variable layer of loose connective tissue consists thick collagen bundles, numerous elastin fibres, blood vessel and provides the connective tissues framework for passage of veins, arteries, lymphatics and Meissener's plexus of nerve. The fibrous connective tissue of submucosa is separating the mucosa from the next layer (3, 5, 10, 75).

Muscularis propria or synonyme (muscularis externa) is a thick muscle coat. The mucularis propria separates the submucosa from the serosa, the outer most layer of the stomach which the muscularis externa has comprised of three layers of smooth muscle; inner oblique layer, middle circular layer and outer longitudinal layer (3, 5, 10, 75).

Serosa is the outermost layer of the stomach, which thin layer and consists of areolar tissues covered by a single layer of squamous mesothelial cell. The serosa a thin covering of loose connective tissue with blood vassels, lymphatics and nerve fibers as shown in Figure 2 (3, 5, 10, 75).

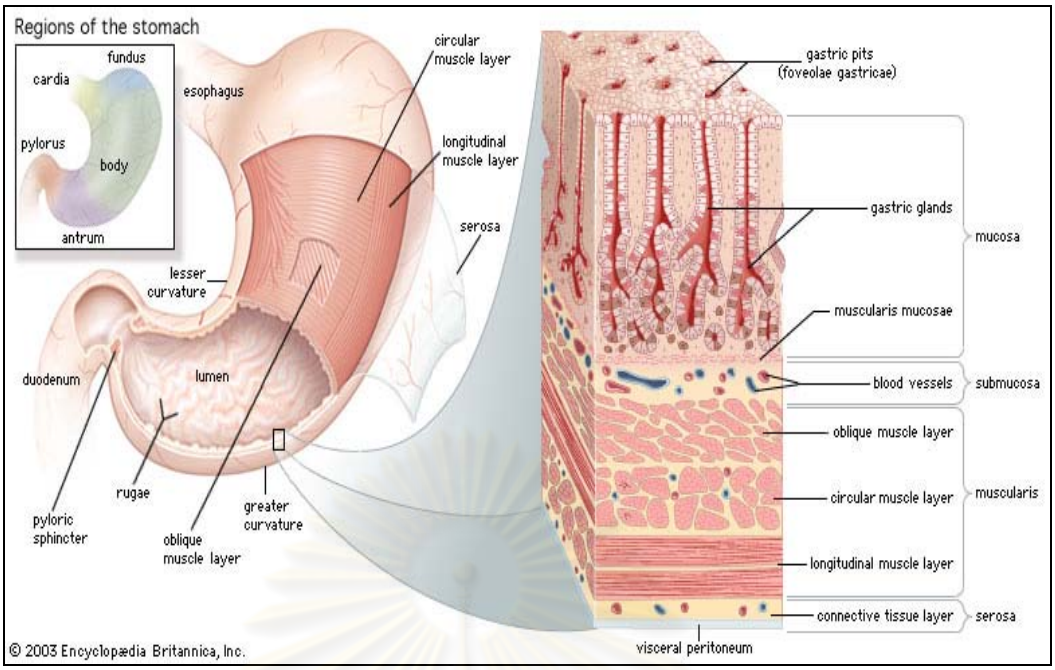


Figure 2. Diagpram showing the principle regions of the interior of the stomach and the microstructure of tissue

DYSPEPSIA

Dyspepsia is a global problem and the management of this condition remains a considerable burden on health care resources (76). Dyspepsia is a common gastrointestinal disorder and prevalence of dyspepsia is approximately 25% or affects more than one fourth of the general population (6, 77, 78). The definition of dyspepsia has varied widely; the most widely applied definition of dyspepsia is the Rome Working Team. The Rome II criteria, dyspepsia is defined as chronic or recurrent pain or discomfort centered in the upper abdomen, however the symptoms of heartburn, acid regurgitation, and belching were excluded from the definition of dyspepsia because of their relation to gastroesophageal reflux disease (GERD) (76, 79).

SYMPTOM OF DYSPEPSIA

Dyspepsia is not one symptom but a compare with several symptoms and different in each patient (7). The symptoms generally refers to abdominal pain, bloating, fullness, nausea, vomiting, (80) early satiety, uncomfortable feeling of fullness after meals, regurgitation, abdominal distension, burping sensation, aching, tenderness, postprandial fullness and anorexia (7).

CAUSES OF DYSPEPSIA

Dyspepsia can be caused by a variety of conditions, including from a number of foods, overeating, ingestion of specific foods such as spicy foods, particularly red and black peppers may cause acute gastric mucosal injury. Coffee (caffeinated or decaffeinated) causes heartburn but its associated to dyspepsia is unproven. Drinking alcohol is challenge to cause of dyspepsia. Medications may cause of dyspepsia by prolong use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin (81) and ibuprofen which 10% to 25% of persons use these agents cause direct gastric mucosal injury. Other medications are general caused of dyspepsia include antibiotics especially macrolides, sulfonamides and metronidazole, potassium supplements, iron, glucocorticoids. Systemic disorders disease such as diabetes mellitus, thyroid disease, hyperparathyroidism, adrenal insufficiency, intra-abdominal malignancy and pregnancy. Chronic disorders, such as rheumatic disorders, are associated with increased gastrointestinal (GI) complaints which medications may be a contributory factor (82). Gastrointestinal tract disease are including gastroesophageal reflux disease (GERD), peptic ulcer disease, cancer of the stomach, gastric or esophageal malignancy, pancreatic, bilinary tract disorder are associated with dyspepsia. Infection of parasites such as *Giardia lamblia* and *Strongyloides stercoralis* may cause of dyspepsia. *Helicobacter pylori* infection is highly prevalent worldwide and is a major cause of diseases occurring in the upper gastrointestinal tract (83).

The patients with chronic dyspepsia for at least 12 weeks have been investigated by esophagogastroduodenal endoscopy but no cause has been found. These groups of patients are labeled as functional dyspepsia or non ulcer dyspepsia or idiopathic dyspepsia (7). The pathophysiology of functional dyspepsia is still poorly understood. There are no organic causes found nor any functional changes observed that correlate with symptom. In present the term of nonulcer dyspepsia is not recommended because peptic ulcer is not the only disease that should be excluded in patients with chronic dyspepsia. Many studies suggest that limited of chronic dyspepsia has a negative impact on health-related quality of life, interferes with daily activities, work, sleep, socializing, eating, drinking, and contributes to emotional stress.

Types of dyspepsia

Dyspepsia are categorized into two major; organic dyspepsia and functional dyspepsia. Organic dyspepsia refers to conditions that have a visible abnormality in the digestive tract. Functional dyspepsia or nonulcer dyspepsia is a common clinical condition characterised with recurrent, chronic and associated with gastrointestinal symptom but normal undergone diagnosis investigation (endoscopy).

GASTRITIS

Gastritis is an inflammation of mucosa in the stomach. The patients, endoscopists, clinicians and pathologists have different concepts of what gastritis which some consider is the symptom complex of the patient. Other determination is an endoscopic appearance of the stomach, however, other the term of gastritis is inflammation of stomach by microscopic evidence (8). Endoscopist is refered to abnormalities of stomach that are visualized by endoscope in term for example, nodular gastritis, subepithelium hemorrhages, granularity and erythema. Gastritis does not specifically refer to the mucosa lesion by used radiographic studies or endoscopy but described by microscopic evidence of inflammation of gastric mucosa in stomach (8). Histologically normal mucosa may appear erythematous when of endoscopy and most the patients endoscopically normal finding gastric mucosa may have histological evidence of inflammation (9). The mechanisms in gastric inflammation are not clear. However, current understanding of gastritis are suggests that an imbalance of aggressive factor and defensive factor that are aggressive factors such as acid production or pepsin, bile, *Helicobacter pylori* infection and defensive factors such as mucus production, bicarbonate, surface hydrophobicity, cell turn over and blood flow (9).

CLASSIFICATION OF GASTRITIS

Gastritis is generally separated on basis of the etiology of mucosal inflammation, into two groups as primary and secondary gastritis. Primary gastritis is uncertain etiology of inflammation which recently study present primary gastritis with bacteria infection, reflux of bile acids from the duodenum into gastric lumen and secondary gastritis is acute inflammation with associated with an acute stress, systemic illnesses, ingestion of ulcergenic agent (9). Some groups of investigator classify by histologic evidence into

active gastritis, chronic gastritis, chronic-active gastritis, and atrophic gastritis which acute gastritis presence of polymorphonuclear lymphocytes but chronic gastritis without polymorphonuclear lymphocytes, chronic-active gastritis is increase number of acute and chronic inflammation , atrophic gastritis occurring from autoimmune (9). The Sydney System for the classification of gastritis emphasized the importance of combining topographical, morphological, and etiological information and clinically useful for diagnosis. Classifications of gastritis by four experts: Rubin, Genta, Appelman, and Correa as shown in Table 1 (84-86)

Table 1. Classification of Gastritis (8)

Chronic Nonspecific	
	Diffuse antral-predominant gastritis with *Helicobacter pylori*
	Multifocal atrophic pangastritis with or without *H. pylori*
	Diffuse corporal atrophic gastritis
Infectious	
	Viral
	Bacterial
	H. pylori and others, including mycobacterial infection
	Fungal
	Parasitic
Granulomatous	
	Crohn's disease
	Sarcoidosis
	Foreign bodies
	Infections
	Tumor-associated
Distinctive Forms	
	Collagenous
	Lymphocytic
	Eosinophilic

Miscellaneous	
	Gastritis cystica profunda
	Graft-versus-host disease
Reactive (Erosive Gastritis)	
	Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) / other medications
	Cocaine
	Stress
	Radiation
	Bile reflux
	Ischemia
	Hiatal hernia
	Trauma (e.g., gastric tubes)

CAUSES OF GASTRITIS

Gastritis can result variety of causes which *H. pylori* infection and nonsteroidal anti-inflammatory drugs (NSAIDs) are the most common causes of gastritis and other cause following the Table 2.

Table 2. Causes of gastritis

1. *Helicobacter pylori*-associated gastritis
2. Bile acid Reflux
3. Stress
4. Exogenous Agent Corticosteroids Nonsteroidal Anti-inflammatory Drugs (NSAIDs) Ethanol
5. Other Exogenous Agent include iron, potassium chloride, calcium salts, antibiotics
6. Infectious Agents, cytomegalovirus, Herpes simplex virus and *Candida albecans*
7. Other causes Crohn's Disease Eosinophilic Gastritis Menetrier's Disease Varioliform Gastritis
8. Autoimmune Causes of gastritis (Atrophic gastritis)

Inflammation of the stomach is considered as acute and chronic gastritis (1, 4). Acute gastritis is an acute mucosal inflammatory process (4). Pathogenesis of acute gastritis is poorly understood and frequecntly associated with heavy used of NSAIDS, particularly aspirin, excessive alcohol consumption, smoking, treatment of cancer chemotherapeutic agent, systemic bacterial or viral infection (e.g., salmonellosis or CMV infection), gastric irradiation, severe stress (e.g., trauma, burns, surgery) (4). As many as 25% of persons take daily aspirin for rheumatoid arthritis development to acute gastritis (4). An acute neutrophilic gastritis (polymorph infiltration is a dominant feature) is characteristic of the initial response to *H. pylori* infection (1). Chronic gastritis is defined as the presence of chronic mucosal inflammation (4). The epithelial changes may become dysplastic and constitute a background for the development of carcinoma. Pathogenesis of chronic gastritis as associated with chronic infection of *H. pylori*, autoimmune, toxic as with alcohol and cigarette smoking, radiation, granulomatous condition, graft-versus-host disease and uremia (4).

PEPTIC ULCER DISEASE (PUD)

Peptic ulcer disease is excavated defects in the gastrointestinal mucosa that is usually acidic and thus extremely painful. The term of peptic ulcer disease is usually used to refer to ulcerations of esophagus called esophageal ulcer, the stomach called gastric ulcer, duodenum called duodenal ulcer, or both. Ulcers have been defined histologically as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa into the submucosa layer or deeper (4) (Figure 3), whereas more superficial necrotic defects are considered erosions of the stomach (11). Ulcers are to be distinguished from erosions, which erosions is epithelial disruption within the mucosa but no breach of the muscularis mucosa (4). Gastric ulcers are generally found in antral mucosa of stomach (Figure 4); duodenal ulcers are found in the proximal duodenum close to the pylorus; esophageal peptic ulcers are found in the squamous epithelium just above the cardioesophageal junction (1). The peptic ulcer disease has progressively increased in the past 50 years. The incidence of duodenal ulcer disease is now the ages of 30 and 60, although may occur in persons of any age. gastric ulcers affect the middle aged and elderly more than the young (87). The sex distribution of duodenal ulcer has shown male predominance today. While gastric ulcer is similar in male and female (87).

In 1979, bacterium *Helicobacter pylori* was discovered by Australian pathologist Robin Warren and Barry Marshall, they isolated the organisms from mucosal specimens from human stomachs and were the first to successfully culture (13). During 1983, pathologist studies that most peptic ulcerations were associated with *H. pylori* infection because it can live in the acidic stomach (88). Prolong used of non steroidal anti-inflammatory drugs (NSAIDs) such as used aspirin for treatment cardiovascular diseases is a side effect of peptic ulcer. Both *H. pylori* and non-steroidal anti-inflammatory drugs (NSAIDs) independently and significantly increase the risk of peptic ulcer and ulcer bleeding. However, may be have other factors are associated with peptic ulcer disease for example, alcohol, diet, diseases associated with peptic ulcer, emotional stress, genetic of host and environment (10, 11).

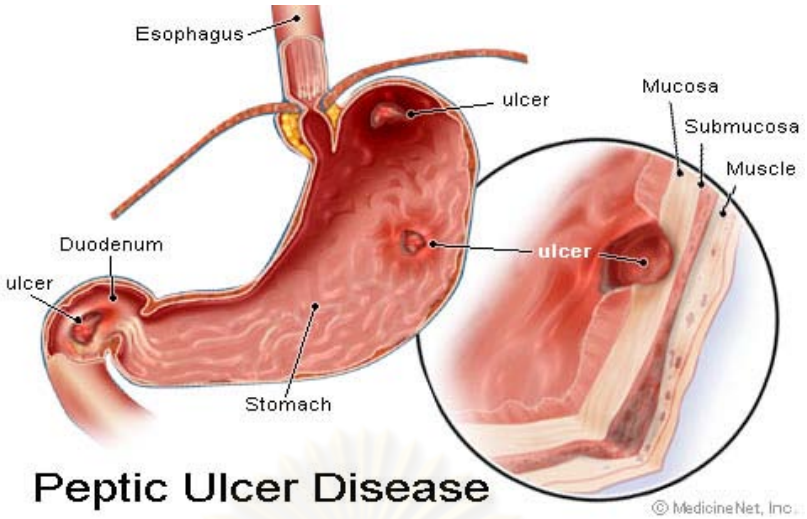


Figure 3. Gastroscopy showing peptic ulcer at body of stomach which defected into the submucosa layer

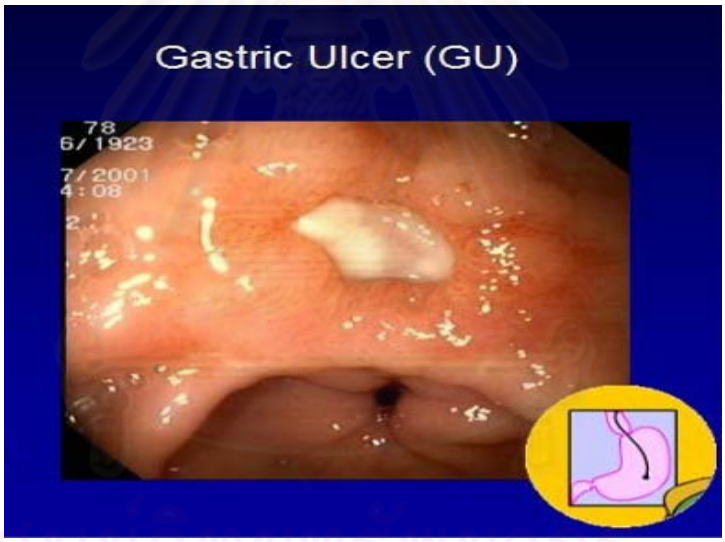


Figure 4. Gastroscopy showing gastric ulcer at antrum of stomach

CAUSE OF PEPTIC ULCERATION

1. *Helicobacter pylori* infection

H. pylori is a helical shaped gram-negative and flagellated bacterium that infects various areas of the stomach and duodenum. The reported *H. pylori* is attaches to gastric epithelial cells in the human stomach and infects about 50% of the world's population (89). They are microorganisms that can thrive in the highly acidic environment of the stomach because it secretes enzymes that neutralize the acid. *H. pylori* produces urease enzyme as catalyzes the breakdown of urea to ammonia and carbon dioxide (88). *H. pylori* can attach on luminal surface of gastric epithelial cell by it use its elf to carbohydrates and sphingolipids. The rate of infection increases with age, so it occurs more often in older people. It also occurs frequently in young people in the developing countries of the world, in countries with poor sanitation and *H. pylori* infected by fecal-oral route. Infection by this bacterium the patients were suffering from chronic gastritis, gastric ulcer and duodenum ulcer. *H. pylori* associated with peptic ulcer disease, both duodenum and stomach which reported 80% of patients with duodenal ulcers and more than 60% of the patient with gastric ulcers are infected with *H. pylori.* However about that less than 20% of individuals infected with *H. pylori* were develop a peptic ulcer (11).

2. Nonsteroidal anti-inflammatory drugs (NSAIDs)

Nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin and arthritis drugs such as ibuprofen can disrupt the protective mucous layer and injury to the gastrointestinal mucosa. Several studies found relative risk of peptic ulcer disease that is associated with the use of NSAIDs (90). NSAIDs induce peptic ulcers can be symptomatic and complicated by GI bleeding, perforation, and/or obstruction. NSAIDs are probably the major mechanism responsible for the acute hemorrhages and erosions. The main risk factors for NSAIDs-related peptic ulcer complications are age, past history, use of higher risk individual NSAIDs, drug dose, concurrent use of corticosteroids. The high concentrations of NSAIDs in cell that cause local toxic effects. NSAIDs cause of pathological changes in gastroduodenal mucosa and stimulate tumor necrosis factor-alpha (TNF-α) and leukotrienes production (11).

3. Other ulcerogenic drugs

A number of drugs other to dispose to damage in duodenum and stomach such as hepatic arterial infusion of 5-fluorouracil for cancer chemotherapy or use potassium chloride in solid condition and cocaine associated with ulceration in gastrointestinal tract. Reports have peptic ulcer associates by use two bisphosphonates, alendronate and risedronate for treatment or prevention of osteoporosis (11).

4. Hypersecretory conditions

Rarely peptic ulcer disease results from disorders that cause of gastric acid secreted in quantities so large by stomach celled hypersecretory condition. Hypersecretion of gastric acid were associated with a syndrome of severe peptic ulcerations. Most defection of epithelial defenses and acid homeostasis affect of peptic ulcer disease that are caused from *H. pylori* infection or NSAIDs used.

5. Cigarette smoking

Cigarette smoking is a risk factor for peptic ulceration and complexity. Healing of peptic ulcerations effects from cigarettes smoking and relapse rate for peptic ulceration. Cigarette smoking may be effect to mucosal protective and aggressive factor (11).

PATHOGENESIS OF PEPTIC ULCER

Peptic ulcers are produced by an imbalance between gastrodeodenal mucosal defense machnisms and the damaging forces as shown in Figure 5 (87). Peptic ulceration occurs when mucosal defenses failure (87). Chronic peptic ulcer are usually less than 20 mm in diameter but may be larger and can exceed 100 mm in diameter (1). Microscopically, the stomach consists of superficial zone of fibrinopurulent exudates, necrotic tissue and polymorph exudates overlying inflammed granulation tissues which merges with mature fibrous (scar) tissue as shown in Figure 5 (1, 87).

H. pylori is major in the pathogenesis of peptic ulcer. However, much interest focused on the mechanisms of *H. pylori*. They do not invade in tissue but it induces immune response. *H. pylori* is increased the production of proinflammatory cytokines such as IL-1, IL-6, IL-8 and tumor necrosis factor-alpha (TNF-α) which these cytokines are produced by mucosal epitheilium cell and it recruits and activates neutrophils (87). Bacterial genes are involevd in causing of epithelial cell injury and induced of inflammation. *H. pylori* is secretes a urease to breaks down urea into toxic form including ammonium chloride and monochloramine (87). *H. pylori* is enhances gastric acid secretion and impairs duodenal bicarbonate production which reducing luminal pH in the duodenum (87). Chronic use of NSAIDs is induced erosion in stomach and suppresses mucosal prostaglandin synthesis.

Environmental factors such as spicy food and caffeine are ulcergenic which contributes to the development or persistence of peptic ulcer. High concentration of alcohol can induce hemorrhagic gastritis but no evidence for peptic ulcer (87). Aspirin is contributing factor in occurs duodenum and especially gastric ulcers. Similarly, other NSAIDs have been the occur of peptic ulcer (87).

Genetic factors are interaction with induce peptic ulcer such as blood-group antigen. The risk of duodenal ulcer is about 30% higher in persons with type O blood than in other types A, B and A B. While, gastric ulcers do not exhibited a greater frequency of blood group O (87).

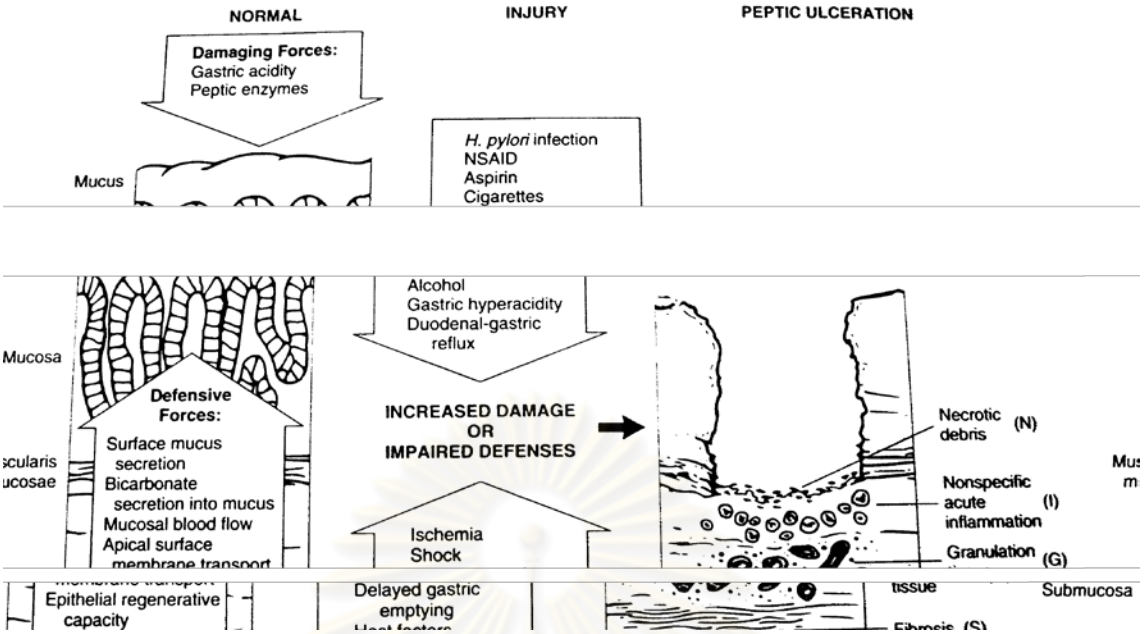


Figure 5. Diagram of causes and defense mechanism gaint peptic ulcer. Peptic ulcer defects of mucosa into the submucosa of stomach. Diagram demonstrating the layers of necrosis (N), inflammation (I), granulation tissue (G), and scar (S) (87)

THE MICROFLORA OF GASTROINTESTINAL TRACT (GIT)

The microflora is microorganism which normally inhabit the healthy human body or other natural environment and not disease causing. The commensal bacteria help to defend against colonization by pathogen. The microflora are categorize into two group; resident flora and transient flora which resident flora is microorganism as present for long period and transient flora present for short period or are present for only a few days, weeks or months before disappearing.

The human gastrointestinal is colonized by 10^{13}- 10^{14} bacteria of 500 different species and subspecies. The human gastrointestinal tract constitutes a complex community of microflora. The microflora establishes after birth and stable of colonize in body of human. The gastrointestinal tract was composed of a diversity of bacteria both pathogenic and non pathogenic. An imbalance of microbial community is major factor for pathology of disease such as inflammatory bowel disease (IBD) and other gastrointestinal disease (91).

GENUS *LACTOBACILLUS*

Scientific classification of *Lactobacillus*

Kingdom	:	Bacteria
Division	:	Firmicutes
Class	:	Bacilli
Order	:	Lactobacillales
Family	:	Lactobacillaceae
Genus	:	*Lactobacillus*

Biology of *Lactobacillus*

Lactobacilli are gram-positive facultative anaerobic bacteria which some strain microaerophilic to anaerobic. They are member of the lactic acid bacteria group. The defined of lactic acid bacteria is large group of beneficial bacteria that produce lactic acid as an end product of the fermentation process. Lactobacilli are straight or curved rods varying length and thickness, cell very from long rod into short rod, sometimes slender or bent rods or diplococci (29). Cell arranged occurring single, in pair, and in chain sometime filamentous or pheomorphic without clubbing or bifid, branching formation. They are non-spore-forming bacteria of the family Lactobacillaceae, and non motile but few strains are motile by peritrichous flagella. Metachromatic granules are predominent in some species, notably *L. bugaricus*, *L. lactis*, and *L. leichmanni.* There are no production of catalase, oxidase, indole and nor eduction of nitrate. The most strain of *Lactobacillus* are vancomycin resistance, some strain vancomycin susceptible (29).

Genus *Lactobacillus* consist of members more than 175 recognized species (www. http://www.dsmz.de/microorganisms/html/bacteria.genus/lactobacillus.html). The G+C content of DNA 32-53 mol%. They are complex nutritional requirement for growth such as amino acid, peptide, vitamin, nucleic acid derivatives, salt, fatty acid or fatty acid esters. Growth is best by anaerobic, facultative anaerobic and microaerobic conditions. Increased of CO_2 concentration (~5%) may stimulate growth. Most strain of lactobacilli growth best at mesophilic temperature about 40ºC and in acidic media optimum (pH 5.5-6.2) (29).

GENERAL CHARACTERISTICS OF THE GENUS *LACTOBACILLUS*

Colony of *Lactobacillus*

Colonies of *Lactobacillus* is growth on agar media are usually small (2-5 mm) in diameter, convex, smooth, glistening and with entire margins. Some species are form rough colonies. They are not pigment production (white colony). In rare case, they are product pigment (yellowish and reddish). Distinctly slimy colonies are only formed by *L. confuses* (30). Growth of *Lactobacillus* in liquid media generally occurs throughout the liquid, but the cells settle soon after growth ceases. The sediment of cell is homogenous and smooth, rarely have slimy or granular. When lactobacilli grown in common media will do not develop attribute odors. However, they contribute to the flavor of fermented food by producing various volatile composite, such as diacetyl, even H_2S and amine in cheese (29).

Cell morphology

The variability of *Lactobacillus* is long rod, straight or slightly crescent rods or curved rods to coryneform and coccobacilli, occurring in single, in pairs and in short or long chain. Rod shape bacteria approximately 0.5-1.6 µm in diameter and long 1-15 µm. The morphology of *Lactobacillus* is differences between species of bacteria (Table 4). The degree of curvature and length of rods are dependent on the age and time of the culture. Some species of gas-producing *Lactobacillus* in liquid media aways shown that a mixture of short and long rods (e.g. *L. fermentum*, *L. brevis*). *L. casei* is short square-ended rod, forming in chain of varying length. Coccobacilli morphology of *Lactobacillus* may confused with *Leuconostoc* and Streptococci. *Lactobacillus* is in general fairly large nonsporing. A few species are motile by peritrichous flagella. Lactobacilli are motile only during isolation but lost after several transfers on artificial media (29, 30).

Nutrition and growth condition

Lactobacilli are extremely meticulous organism and adapted to the complex of organic compound. They require carbohydrates as to the carbon source and energy which not only carbohydrates but also amino acid, nucleotides and vitamins. Thiamine is only

nessesary for the growth of nearly all the heterfermentative lactobacilli but not those that are homofermentative, while Nicotinic acid and pantothenic acid are exception of a few strains. The requirement of *p*-aminobenzoic acid, puridoxal phosphate, roboflavin, folic acid is related of among the various of lactobacilli. Vitamins B12 and Biotin are only necessary for a few species of lactobacilli. The pattern of the amino acid requirement for lactobacilli are differs among species (29).

Culture characteristic

All media for isolation of lactobacilli are complex. The various requirement of essential nutrients are normally met when the media contain fermentable carbohydrate, peptone, meat and yeast ex tract. Supplementation of medium with tomato juice, manganese, acetate and oleic acid ester, espectially tween 80 are stimulates the growth of many lactobacilli (30). The media generally used for cultivation of lactobacilli is de Man, Rogosa and Sharpe (MRS) at pH 6.2-6.4. The MRS media formulation was developed by de Man, Rogosa and Sharpe to replace the tomato juice medium and the meat extract tomato juice medium which MRS medium is supporting good growth of lactobacill (92). MRS agar is based on for the enrichment, cultivation and isolation of *Lactobacillus* species from all types of materials. The compounds of MRS are contained sodium acetate, polysorbate (Tween 80), magnesium and manganese ions which are known to act as special growth factors for lactobacilli. MRS media can applied by changing the concentration of inhibitors, pH and temperature or time for incubation (29, 30, 92)

Growth characteristic

Lactobacilli can culture in laboratory which most strains of *Lactobacillus* growth in facultative anaerobic, a few are strict anaerobe, and some strains grow under microaerophilic condition. Lactobacilli incubated at 37ºC for 24 -48 h. Most of lactobacilli will grow in air but grow best in an atmosphere lacking oxygen but required carbon dioxide (CO_2). Lactobacilli are grow best in acidic media with an initial of pH 6.4-4.5, the growth of lactobacilli ceases when pH 4.0-3.6 is reached, depending of the species and strain. Lactobacilli are grow in acidic media optimum (pH 5.5 -6.2) and growth rate is reduced when nutral or increase alkaline reactions. The temperature at which growth occur varies with species and strains. Most lactobacilli growth best at

mesophilic temperature approximate 40ºC which optimum temperature for growth 30-40ºC, some strains growth below 15ºC and some strains may be growth an upper 55ºC, they are celled thermophilic lactobacilli as show in Table 4. The thermobacteria are usually large, thick and often filamentous (29).

Metabolism characteristic

The main of fermentation pathways are catagorized into two group; homofermentative and heterofermentative. In the group of homofermentative species, glucose is broken down to lactic acid almost exclusively by the Embden-Meyerhof pathway. The heterofermentative species, possess the 6-phosphogluconate pathway which the end products are CO_2, acetic acid, ethanol and lactic acid. Heterofermentative lactobacilli are distinguished from homofermentative by their ability to produce differs end produce (29, 30). *Lactobacillus* species can be divided into three groups by carbohydrate fermentation. The group of obligately homofermentative lactobacilli are including *L. acidophilus*, *L. delbrueckii*, *L. helveticus*, *L. salivarius* and *L. lactis* as shown in Table 3. Obligately heterofermentative lactobacilli are consists of *L. brevis*, *L. buchneri*, *L. fermentum*, *L. reuteri*. However, *L. casei*, *L. paracasei*, *L.curvatus*, *L. rhamnosus*, *L. plantarum* and *L. zeae* were closely related taxonomic group within the facultatively heterofermentative lactobacilli (Table 3) (93).

Table 3. The major of lactobacilli for fermention

Obligately homofermenter	Facultative homofermenter	Obligately heterofermenter
Lactobacillus acidophilus	*Lactobacillus bavaricus*	*Lactobacillus brevis*
Lactobacillus lactis	*Lactobacillus casei*	*Lactobacillus buchneri*
Lactobacillus delbrueckii	*Lactobacillus coryniformis*	*Lactobacillus cellobiosus*
Lactobacillus leichmannii	*Lactobacillus curvatus*	*Lactobacillus confusus*
Lactobacillus salivarius	*Lactobacillus plantarum*	*Lactobacillus reuteri*
Lactobacillu helveticus	*Lactobacillus sake*	*Lactobacillus fermentatum*
	Lactobacillus paracasei	*Lactobacillus sanfrancisco*
	Lactobacillus rhamnosus	

Genome Structure

The genome sequences of a number of different species of *Lactobacillus.* The present time the genomes of *L. johnsonii* NCC 533, *L. plantarum* WCFS1, *L. acidophilus* NCFM , *L. gasseri* ATCC 33323, *L. delbrueckii subsp. bulgaricus* ATCC BAA-365, *L. casei* ATCC 334, and *L. brevis* ATCC 367 have been completely genome sequenced.

In 2002 Michiel Kleerebezem *et at.* study sequence of the chromosome of *L. plantarum* WCFS1 was found that contains a single, circular chromosome of 3,308,274 bp and G+C content of the chromosome is 44.5% and was found to contain two small, cryptic plasmids (2,365 and 1,917 bp) and a larger plasmid (36,069 bp) encoding genes involved in conjugal plasmid transfer and several other (94). Eric Altermann *et al*. they suggested the complete genome of *L. acidophilus* NCFM consisted of 1,993,564 bp long with 34.71% G+C content (95). Previous study *L. johnsonii* NCC 533 is a member of the acidophilus group of intestinal lactobacilli. The genome of *L. johnsonii* NCC 533 is about 1,992,676 bp long and is G+C content of 34.6% (96). *L. gasseri* ATCC 33323, a strain of human origin and a native species found commonly in the gastrointestinal tract. The plasmid-free genome was 1,894,360 bp in size and predicted to encode 1,810 genes and GC content was 35.3% which similar to the GC content of its closest relatives, *L. johnsonii* NCC 533 (34%) and *L. acidophilus* NCFM (34%) (97).

HISTORICALPERSPECTIVE OF *LACTOBACILLUS*

Lactobacilli are found in several environments shown that in the Table 4.The type species of the genus *Lactobacillus*, *L. delbrueckii* was original isolated from milk by Leichmann (1896) and similar bacilli was observed in the vaginal secretion of women by Doderlein (1892). In 1900 Moro culture culture slender rod, *L. acidophilus* from the feces of breast-fed babies. Lactobacilli isolated from milk and cheese, dairy products and dairy were name *L. casei* by Orla-jensen (1904)**.** Lauer and Kandler (1980) isolated *L. gasseri* from human mouth, vagina and from the intestinal tract of man**.** Heinemann and Hefferan (1909) isolated lactobacilli from human saliva, soil, gastric juice and various foods. In 1953 Rogosa, Wiseman, Michell isolated *L. salivarius* from mouth and intestinal tracts which simillar with *L. murinus* isolated by Heijenoort *et al* (1982) (29, 30).

Table 4. List of the species of the genus *Lactobacillus* (29)

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
Lactobacillus delbrueckii	Leichmann (1896) Doderlein (1982)	Rod with rounded ends, 0.5-0.8x2-9 µm.	occurring singly and in short chain	*L. delbrueckii, L. bulgaricus, L. lactis L. leichmannii*	+	49-51	milk
L. delbrueckii subsp. delbrueckii	Leichmann (1896)	Rod with rounded ends , 0.5-0.8x2-9 µm.	occurring singly and in short chain	*L. delbrueckii, L. bulgaricus, L. lactis L. leichmannii*	+	49-51	Plant material fermented at high temperatures
L. delbrueckii subsp. bulgaricus	Orla-Jensen(1919) Weiss *et al*. (1984)	Rod with rounded ends , 0.5-0.8x2-9 µm.	occurring singly and in short chain	*L. delbrueckii, L. bulgaricus, L. lactis*	+	49-51	Yoghurt and cheese
L. amylophilus	Nakamura and Crowell (1981)	Thin rod, 0.5-0.7x2-3 µm.	occurring singly and short chains	-	-	44-46	Swine waste-corn fermentation

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. acidophilus	Moro (1900) Hansen and Mocquot (1970)	Rod with rounded ends 0.6-0.9x1.5-6 µm.	occurring singly, in pair and in short chains	-	+	32-37	The feces of breast-fed babies, the intestinal tract of human and animal,
L. amylovorus	Nakamura (1981)	Rods, 1x3-5 µm.	occurring singly and short chains	*L. acidophilus, L. leichmannii L. amylophilus*	+	40-41	Cattle waste-corn fermentation
L. animalis	Dent and Williams (1983)	1.0-1.2x3-6 µm.	occurring singly or in pair	*L. murinus*	+	41-44	Dental plaques and alimentary canal of animal
L. crispatus	Brygoo and Aladame (1953) Moore and Holdeman (1970)	Straight to slightly curved rod, 0.8-1.6x2.3-11 µm.	occurring singly and short chains	*L. acidophilus*	+	35-38	Human feces ,vagina and buccal cavities, crops and ceca of chicken
L. gasseri	Lauer and Kandler (1980)	Rod with rounded ends	occurring singly and in chains	*L. acidophilus L. crispatus*	+	33-35	Human mouth and vagina

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
		0.6-0.8x3-5 µm.					intestinal tract of man and animal
L. helveticus	Orla-Jensen (1919) Bergey *et al*. (1925)	-	-	*L. jugurti*	+	37-40	Sour milk, cheese starter cultures
L. johnsonii	Gasser,Mandel and Rogasa (1970)	Rod with rounded ends 0.6-0.8x2-4 µm.	occurring singly and in short chains	-	+	35-37	Human vaginal discharge and blood clot
L. ruminus	Sharpe,Latham,Garvie, Zirngbl and Kandler(1973)	Rods , 0.6-0.8x3-5 µm.	occurring singly, in pairs and in short chains	-	-	44-47	Rumen of cow and sewage
L. salivarius subsp.salivarius *L. salivarius subsp.salicinius*	Rogosa ,Wiseman, Mitchell and Dilraely(1953)	Rod with rounded ends, 0.6-0.9x1.5-5 µm.	occurring singly and in chains varying length	-	+	34-36	Mouth and intestinal tract of man and hamster

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. sharpeae	Weiss,Schillinger ,Lacternser and Kandler (1982)	Rod with rounded ends, 0.6-0.9x1.5-5 µm.	Long characteristicall y wrinkled chain	-	-	53	Municipal sewage
L. alimentarius	Reuter (1983)	Short, slender rod, 0.6-0.8x1.5-2.5 µm.			-	36-37	Marinated fish products, meat products and sour dough
L. bavaricus	Stetter and Stetter (1980)	Rod with rounded ends 0.8-1.0x2-7 µm.	occurring singly and in short chains	*L. sake* *L. curvatus*	-	41-43	Sauerkraut and fermented cabbage leaves
L. casei subsp.casei	Orla-Jensen (1916)	Rods, 0.7-1.1x2-4 µm.	In chains	-	-	45-47	Milk and cheese, dairy products and dairy environment, human intestinal tracts, mouth, vagina

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. casei subsp.pseudoplantarum	Abo-Elnaga and Kandler (1965)	Rods, 0.7-1.1x2-4 µm.	In chains	-	-	45-47	Milk and cheese, dairy products and dairy environment, human intestinal tracts, mouth, vagina and sewage
L. casei subsp.rhamnosus	Hensen (1968)	Rods, 0.7-1.1x2-4 µm.	In chains	-	-	45-47	Milk and cheese, dairy products and dairy environment, human intestinal tracts, mouth, vagina and sewage
L. casei subsp.tolerans	Abo-Elnaga and Kandler (1965)	Rods, 0.7-1.1x2-4 µm.	In chains	-	-	45-47	Milk and cheese, dairy products and dairy environment, human intestinal tracts,mouth,vagina

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. coryniformis	Abo-Elnaga and Kandler (1965)	Short, often coccoid , rods , 0.8-1.1x1-3 µm.	occurring singly, in pairs and in short chains	-	-	45	Silage, cow dung, dairy barn air and sewage
L. coryniformis subsp. coryniformis	Abo-Elnaga and Kandler (1965)	Short, often coccoid ,rods , 0.8-1.1x1-3 µm.	occurring singly, in pairs and in short chains	-	-	45	Silage, cow dung, dairy barn air and sewage
L. coryniformis subsp. torquens	Abo-Elnaga and Kandler (1965)	Short, often coccoid ,rods , 0.8-1.1x1-3 µm.	occurring singly, in pairs and in short chains	-	-	45	Silage, cow dung, dairy barn air and sewage
L. curvatus	Abo-Elnaga and Kandler (1965)	Curved, bean–shape rods, 0.7-0.9x1-2 µm.	Occurring in pairs and in short chains	*L. sake, L. cuvatus*	-	42-44	cow dung, milk, salage ,sauerkeaut and meat products

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. homohiochii	Kitahara,Kaneko and Goto (1957)	Rods, with rounded ends, 0.7-0.8x2-4 µm.	-	*L. sake*	-	35-38	Spoiled sake
L. maltaromicus	Miller, Morgan and Libbeu (1974)	Slender rod of varying large	Long chain	-	-	36	Milk products
L. murinus	Hemme, Raibaud, Duckuzeau ,Galpin, Sicard and van Heijenoort (1982)	Rods, with rounded ends, 0.8-1x2-4 µm.	In chain	-	-	43-44	Intestinal tract of mice and rats
L. plantarum	Orla-Jensen (1919)	Straight, rod with rounded ends, 0.9-1.2x3-8µm.	occurring singly, in pairs and in short chains	-	-	44-46	Dairy products and environment, silage , pickled vegetables, cow

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
							dung and human mouth, intestinal tract and stool ans sewage
L. sake	Katagiri,Kitahara and Fukami (1934)	Rods with rounded ends, 0.6-0.8x2-3 µm.	Occurring in pairs and in short chains	-	-	42-44	Sake starter , fermented plant material, meat products
L. bifermentans	Kandler, Schillinger and Wiess (1983)	Irregular rods , 0.5-1x1.5-2 µm.	Occurring singly ,in pair or irregular short chains	-	-	45	Spoiled Edem and Gouda cheese
L.brevis	Orla-Jensen (1919)	Rods with rounded ends, 0.7-1x2-4 µm.	Occurring singly and in short chains	*L. hilgardii, L. kefir, L. confuses, L. collinoides*	-	44-47	Milk, cheese, sauerkraut, sour dough, feces, mouth gastrointestinal tract of human and rats

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. buchneri	Henneberg (1903)	Rods with rounded ends, 0.7-1x2-4 µm.	Occurring singly and in short chains	-	-	44-46	Milk, cheese, fermenting plant material and mouth of human
L. collinoides	Carr and Davies (1972)	Rods with rounded ends, 0.6-0.8x3-5 µm.	Occurring singly, in palisades and irregular clumps	-	-	46	Cider
L. confusus	Holzapfel ans Kandler (1969)	Short rods , 0.8-1x1.5-3 µm.	Occurring singly,rarely in short chains	-	+	45-47	Sugarcane and carrot juice, raw milk, saliva and waste matter
L. divergens	Holzapfel and Gerber (1984)	Rods with rounded ends, 0.5-0.7x1-1.5 µm.	occurring singly, in pairs and in short chains	-	-	33-35	Vacuum packaged, refrigerated meat

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. fermentum	Berjirinck (1901)	Rods ,0.5-0.9 µm. thick and highly variable length	occurring singly and in pairs	*L. cellobiosus*	+	52-54	Yeast, milk products, sour dough , fermenting plant material, manure, sewage, mouth and feces of human
L. fructivorans	Charlton, Nelson and Werkman (1934)	Rods with rounded ends, 0.5-0.8x1.5-4 µm.	Occurring singly, in pairs and in chains	*L. trichodes* *L. heterohiochii*	-	38-41	Spoiled mayonnaise, salad dressings, and vineagar preserves, spoiled sake, dessert wines
L. fructosus	Kodama (1956)	Rods , 0.5-0.8x2-4 µm.	Occurring singly, in pairs and in short chains	-	-	47	Flowers

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. halotolerans	Kandler, Schillinger and Weiss (1983)	Irregular ,short or coccoid rods, 0.5-0.7x1-3 µm.	Coiling chains, clumping	-	-	45	Meat products
L. hilgardii	Douglas and Cruess (1936)	Rods with rounded ends, 0.5-0.8x2-4 µm.	occurring singly , in short chains ,long filaments	*L. brevis, L. desidiosus ,L. reuteri*	-	39-41	California table wine
L. kandleri	Holzapfel ans van Wyk (1983)	Partly irregular rods, 0.7-0.8x1-5 µm.	Occurring singly, in pairs, seldom in short chains	-	-	39	Desert and spring
L. kefir	Kandler and Kunath (1983)	Rods with rounded ends, 0.6-0.8x3-15 µm.	Tendency to from chains of short rod or long filaments	-	-	41-42	Kefir grains and kefir

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. minor	Kandler ,Schillinger and Weiss (1983)	Irregular ,short rod, 0.6-0.8x1.5-2 µm.	Occurring in pair and short chain	-	-	44	The sludge of milking machines
L. reuteri	Kandler ,Stetter and Kohl (1982)	Slightly irregular rods, bent rod , 0.7-1x2-5 µm.	Occurring in pair, short chain and small clusters	-	+	40-42	Feces of human and animal and meat products
L. sanfrancisco	Weiss and Schillinger (1984)	Rods with rounded ends, 0.6-0.8x2-4 µm.	Occurring singly and in pairs	*L. acidophillus* *L. helveticus* *L. brevis* *L. confusus*	-	36-38	Sour dough
L. vaccinostercus	Okada, Suzuki and Kozaki (1983)	Rods with rounded ends, 0.9-1x1.5-2.5 µm.	Occurring mostly in pair	-	-	36	Cow dung
L. viridescens	Niven and Evens (1957)	Small, irregular rods, 0.7-0.9x2-5 µm.	Occurring singly and in pairs	-	-	41-44	Discolored cured meat products and pasteurized milk

Species	First isolate	Rod shape and size	arrangement	DNA/DNA homology	Growth at 45 ºC	Mol % G+C	Original isolated
L. catenaforme	Eggerth (1935)	Small, slightly irregular rods, 0.7-0.9x2-5 µm.	Occurring in chain	-	+	31-33	Human feces,intestinal and pleural infections
L. minutus	Houduroy, Ehringer, Urbain,Guillot and Magrou (1937)	Small,elliptical rods,	Occurring singly,in pair and short chains	-	-	45	Abscesses and wounds

ECOLOGY OF *LACTOBACILLUS*

Lactobacilli have been detected in diverse environments. They are used in the production of foods prepared by means of lactic acid fermentation such as dairy products, fermented vegetables, fermented meats, and sourdough bread. They are found in plant material such as in foodstuffs, silage and agriculture products (30). Some species of *Lactobacillus* is used industrially for the production of milk and dairy, cereals produce such as beer, wine and cider. *L. casei* is remarkably adaptive species, and may by isolated from row and fermented dairy products and industrially application for as acid producing starter cultures for milk fermention. Several previous study, milk products containing *L. acidophilus* has the potential for preventing or controlling intestinal infections, helping control serum cholesterol levels, enhancing lactose digestion and absorption in people who are lactose intolerant, and exerting anticarcinogenic activity (98).

Lactobacilli are commonly associated with the body of humans and animals. They are microflora in the oral cavity, gastrointestinal tracts and vagina (33, 34). The oral cavity presented diverse microflora due to the different anatomical sites and exposure to the external environment. Lactobacilli were found in oral cavity. They are habitat on teeth, in saliva, the tongue and the hard palate in humans (99). Previous study frequency *Lactobacillus* in oral cavity which found that in saliva and tongue with 90% and 50% respectively (100). The species of *Lactobacillus* is predominant in oral cavity, a wide range such as *L. acidophilus*, *L. casei*, *L. paracasei L. plantarum*, *L. salivavius*, *L. fermentum*, *L. rhamnosus*, *L. buchneri* and *L. brevis* (34, 101, 102). *L. murinus* is found in the oral cavity of mice (103).

Lactobacilli are microflora in vagina of human. The role of lactobacilli in the maintenance of vaginal health was first recognized by Doderlein in the late 1800. Lactobacilli are dominant in this habitat at 10^7 to 10^8 CFU/g of vaginal fluid in healthy premenopausal women. Previous study vaginal *Lactobacillus* of healthy women in the late first trimester of pregnancy. The most frequently occurring species were *L. crispatus* and *L. gasseri* (104) followed by *L. jensenii* and *L. rhamnosus*. Pregnant women with a *Lactobacillus*-predominant microflora have a decreased incidence of preterm delivery, chorioamnionitis and postpartum infections (105). Previous studies indicated that

colonization of *L. vaginalis*, *L. crispatus*, *L. jensenii*, *L. gasseri* and *L. iners* are the most common species of vaginal lactobacilli (106). The study characterized human *Lactobacillus* isolates for their capacity to interfere with the growth of *Candida albicans* using an agar-overlay method and identified *L. fermentum* Ess-1 was significantly inhibited the growth of *C. albicans* which vaginal candidiasis pathogens (48). In 2007 Falagas *et al.* study clinical trials was showed of *L. acidophilus* or *L. rhamnosus* GR-1 and *L. fermentum* RC-14 reduced the recurrences of bacterial vaginosis (BV) (49, 50). Therefore, the colonization of the human vagina by *Lactobacillus* species is important because a clinical study suggested that the presence of H_2O_2- generating *Lactobacillus* strains decreases bacterial vaginosis (BV). *L. crispatus* strains have the potential to be developed of vaginal probiotics (104).

The human gastrointestinal tract harbour a complex microflora of over 500 different species and strains (107). The human gastrointestinal tracts are including stomach, small intestine and large intestine. Lactobacilli habitat in gastrointestinal tracts and widely considered of beneficial role including immunomodulation, interference with enteric pathogen and plays an important role in normal gut function and maintaining host health. The fetal gut is sterile, and colonization with bacteria is sustained by contact between the child and its environment, depending on the mode of delivery (108, 109), hygiene levels, medication and type of feeding, the use of antibiotics or other medicine (110), and differences in gut microflora composition occur between breast- and formula-fed infants (107, 111). The evidence of *L. salivarius* CECT 5713 was originally isolated from feces of a one-month-old breast-fed infant which suggested that the gut microflora of breast-fed infants reflects that of the maternal breast milk (112). The reported *Lactobacillus* is present in gastrointestinal tracts of mice and it important role in healthy animals and it is colonize in stomach and intestine of mice after birth by adhere on epithelial cells of host and they are stable in intestinal microflora of the animals (32). The various species of *Lactobacillus* in gastrotintestinal tracts are consists of *L. salivarius*, *L. cesei*, *L. plantarum*, *L. fermentum*, *L. acodophilus*, *L. gasseri*, *L. brevis* and *L. reuteri* (32). *L. reuteri* has been rarely in gastrointestinal tract which recent studies by culture and molecular method.

The human stomach has been observed as an inhospitable environment for microorganism because of acidic conditions and other antimicrobial factors. *Lactobacillus* species could be found in the stomach, which characterized by pH 2.2-2.4. The population of bacteria in stomach about 10^3 CFU/ml (69) which is few bacterial species which are all resist to the hydrochloric acid in saliva envionment and gastric acid-tolerant and consist of lactobacilli and streptococci. Previous study reported bacterial diversity within the human gastric mucosa by using 16S rDNA were assigned to majority of the phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria* (70). Previous study the composition of the *Lactobacillus* microflora from mucosa of human stomach and recognized new novel lactobacilli. Lactobacilli analysed by 16SrRNA sequencing into two belong to the *L. reuteri* and the other two to the *L. delbrueckii* subgroup of *Lactobacillus* which names *L. gastricus* sp. nov., *L. antri* sp. nov., *L. kalixensis* sp. nov. and *L. ultunensis* sp. nov (35). The microflora can interfere adherence of the pathogen in stomach. The reported *L. acidophilus* has an inhibitory effect on *H. pylori* isolated from peptic ulcer patients and could enhance antibiotic therapy for *H. pylori* eradication (71, 72). Prior study *Lactobacillus* was considered to be indigenous in stomach of rat, mice and, pig (73). *Lactobacillus* was colonized on surface of the squamous keratinized epithelium cell in part of stomach. Study *Lactobacillus* can isolate from biopsy of stomach of conventional rats (74).

The small intestine represents a transitional between the sparsely population of stomach and abundant of the bacterial microflora of colon. The proximal small bowel (duodenum) has concentration rang 10^3-10^4 CFU/ml which similar of the stomach. Jejunal microflora is similar of that of the stomach. The predominant species are lactobacilli, streptococci and stephylocci (69). The microflora of ileum was complex similar to that of the large intestine. Lactobacilli are also common inhabitants of the large intestine of humans which can culture from human feces. Numerically predominant genera inhabiting the large bowel attain population levels of about 10^{11} to 10^{12} CFU/g of fecal samples (101, 113, 114). Predominant species in large intestine are including lactobacilli, bifidobacteria and enterococci. Previous study molecular analysis was used ribotyping and pulsedfield gel electrophoresis (PFGE) of genomic DNA of the microflora of human feces which examined at the level of bacterial strains. Bifidobacterial and *Lactobacillus* populations were characteristic of the particular human (115, 116).

The study species of *Lactobacillus* from intestine biopsies was found that *L. rhamnosus*, *L. salivarius*, and *L. acidophilus*-like which *L. acidophilus*- like isolates could not differentiated from *L. cribpatus*, *L. jensenii* and *L. gasseri* (34). Tannock *et al.* study change composition microflora of humans feces by probiotic containing *L. rhamnosus* DR20 for 15 months. They found that species of lactobacilli in human feces were difference on individual of human which *L. acidophilus*, *L. jonhsonii*, *L. crispatus* (104), *L. murinus*, *L. salivarius*, *L. brevis, L. casei*, *L. plantarum*, *L. delbrueckii* and *L. gasseri* regularly detected in the feces of human. Moreover, these study showed that *Lactobacillus* including *L. brevis, L. casei*, *L. plantarum*, *L. delbrueckii* were indicated (allochthonous) strains. Allochthonous strain is transient strains that originated in food and passed through the intestinal tract strains and can detect in feces. In contrast *L. ruminis* or *L. salivarius* subsp. *salivarius* strains was detected as the persistant for several months which these strains could be referred to as (autochthonous) strains (116). Prior study fermented milk containing *L. acidophilus* and *Bifidobacterium* was performed in 6 healthy volunteers who ingested which a large number of living *L. acidophilus* and *Bifidobacterium* pass through the upper gastrointestinal tract into the colon (117).

The defined and categorized the gastrointestinal microflora into two types, autochthonous microflora (indigenous microflora) and allochthonous flora (transient microflora) (91). Indigenous is strains inhabiting a place or region from earliest times, organism that colonize specific niches in human body. The genera of *Lactobacillus* were suggested that the species *L. crispatus*, *L. gasseri*, *L. reuteri* (118), *L. ruminis*, and *L. salivarius* are truly autochthonous to the human gastrointestinal tract (33, 116). *L. gasseri and L. reuteri* were occasionally present in stomach and proved to be predominant autochthonous both species were found in infants and adult (97). However, some species of *Lactobacillus* may be is allochthonous microflora species derived from food or oral cavity and an altered to microflora but can persists in some niches are not filled than native microflora (119). Allochthonous microflora lactobacilli are present into the gastrointestinal tract because they are ubiquitous in nature. These lactobacilli are transferred from food into the stomach and small intestine into the large bowel and can collected from human faeces. Furthermore, suggested that some *Lactobacillus* species found in the gastrointestinal tract may be originates from the oral cavity (120).

The presence of lactobacilli in digestive tract system has historically the beginning Elie Metchnikoff, which affluence of experiments have reported the use of selected microorganisms, mainly belonging to the lactic acid bacteria family, for the prevention or treatment of a variety of pathological situations (96). *Lactobacillus* is lactic acid bacteria which can produce lactic acid makes environment acidic which inhibits the growth of bacterial pathogen. Recently *Lactobacillus* is increasingly used as probiotics in industial of fermented foods and pharmaceuticals. The selection of lactobacilli strain is performed in the intestinal microflora of man, infants and adults were sampling from faeces, upper gastrointestinal tracts including oral cavity and different parts of gastrointestinal tract including stomach, caecum and colon were sampling from biopsy.

Antimicrobial compounds of *Lactobacillus*

Lactic acid bacteria also produce antimicrobial substances are including organic acid (lactic acid, acetic acid and propionic acid), ammonia, hydrogen peroxide (H_2O_2), fatty acids, carbon dioxide (CO_2) and other metabilites. Many of these metabolites are bacteriocin, siderophore, benzoic acid and reuterin. The lactic acid they produce is effective in inhibiting the growth of other bacteria that may decompose or spoil the food.

Organic acid

The ability *Lactobacillus* as lactic bacteria group is produced acid by carbohydrate fermentation which including lactic acid, acetic acid, propionic acid and ethanol. Thus the environment of the digestive tract is acidified and the acidosensitive pathogens cannot exert their effect. It also characteristically produces an acidic environment which can inhibit growth of other organisms which cause genitourinary tract infections. Indigenous intestinal microflora such as *L. paracasei* and *L. plantarum* were antagonistic *Clostridium difficile* which antagonistic activity was strain-specific and seemed to correlate with lactic acid production (42).

Hydrogen peroxide

Hydrogen peroxide is produced by *Lactobacillus*. The effect of H_2O_2 has been attributed to its strong oxidizing effect on the bacterial wall. Several species of lactobacilli produce H_2O_2 in an oxygen atmosphere are including *L. acidophilus* and

L. delbrueckii ssp. *bulgaricus*. P roduction of H_2O_2 is be lieved to be be neficial f or prevention t he growth of pa thogen. Previous s tudy of hum an intestinal is olate *L. johnsonii* NCC 533 produced H_2O_2 was e ffective i n ki lling t he m odel pa thogen *Salmonella enterica* serovar T yphimurium S L1344 *in vitro* (121). The s everal studies *Lactobacillus* can i nhibit the growth of ba cterial vaginasis (122). The killing properties of h ydrogen p eroxide e xerted t oward *Escherichia coli* and *Candida albicans* were l ess prominent than these of the s upernatants of c ultures of *Lactobacillus* strains pr oducing H_2O_2.

Diacetyl (2, 3-butanedione)

Diacetyl is produce from fermentation of citrate by lactobacilli. The requirement of citrate for the production of diacetyl and acetoin is well recognized in certain species of l actic a cid bacteria. The a ntimicrobial a ctivity of di acetyl w as evaluated against *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* (123). The antimicrobial activity of diacetyl was antagonized by glucose, acetate, and Tween 80 but not b y gluconic a cid. As an antimicrobial ag ent, diacetyl w as cl early more ef fective against g ram-negative ba cteria, yeasts, a nd m olds t han a gainst gr am-positive ba cteria (124).

Bacteriocins

Bacteriocins are i nhibitory substances t hat a re ge nerally produced b y gr am positive ba cteria. Its ha ve hi gh m olecular w eights and susceptible t o protease and their spectrum of antimicrobial activity related with species of bacteria (125). The inhibitory substance as produced by *Lactobacillus* has a low molecular weight and active against a broad spectrum of gram-positive and gram-negative bacteria. Bacteriocins are believed to be impor tant in the a bility o f la ctic a cid bacteria to compete in non-fermentative ecosystems such as the gastrointestinal tract.

Reuterin

Reuterin (3-Hydroxypropionaldehyde) is a newly discovered, broad-spectrum antimicrobial substance which produced by *L. reuteri* during fermentation of glycerol. *L. reuteri* a distinctive species originally described by Gerhand Reuter (1980) is a prominent member of the heterofermentative *Lactobacillus* species in the gastrointestinal tract of human and animals (126). Axelsson *et al.* (1989) reported the *L. reuteri* converted glycerol into broad–spectrum antimicrobial by anaerobic resting cells under physiological conditions of temperature and pH. Preliminary investigations indicate that of leuterin its a low molecular weight, neutral and water soluble compound, nonprotein material that has antibacterial, antimycotic, and antiprotozoal activity. The ability of reuterin as inhibit the growth of pathogen several bacterial genera including *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *Clostridium* and *Staphylococcus* as well as yeasts, fungi, and protozoa (126-129). In vitro studies on reuterin excretion by *L. reuteri* was synthesized under environmental conditions similar to those that exist in gastrointestional tract and was stimulated by contact with other bacteria found in gut such as *E. coli*, *Samonella typhimurium*, *Shigella*, *Proteus*, *Pseudomonas florescens*, *Clostridiums sporogenes*, *Streptococcus cremoris*, *Staphylococcus epidermidis*, *Bacillus megaterium* (130). As shown in Table 5.

Table 5. *Lactobacillus* and action of antimicrobial compound from *Lactobacillus*

Antimicrobial compound	Source	Action	Reference
Organic acid	*L. johnsonii* NCC 533	kill pathogen *Salmonella enterica* serovar Typhimurium SL1344 *in vitro*	(121)
Hydrogen peroxide	*L. delbrueckii* subsp. *lactis* T31 *L. paracasei* and *L. plantarum*	against food-borne pathogens, decrease in *Listeria* viability antagonistic *Clostridium difficile*	(131) (42)

Antimicrobial compound	Source	Action	Reference
Bacteriocin	*L. salivarius BGHO1*	against growth of *S. mutans*, *S. pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Micrococcus flavus*, and *Salmonella enteritidis*	(132)
	L. acidophilus AA11	against spoilage microorganisms (i.e. *Staphylococcus aureus* and Bacillus cereus) and pathogens (i.e. *E. coli*, *Salmonella sp.* and *Shigella sp.*	(133)
	L. gasseri LA39	inhibit food-borne pathogenic bacteria by gassericin A produced	(134)
Reuterin	*L. reuteri*	inhibits the growth of gram-positive and gram-negative bacteria as well as yeasts, fungi, and protozoa	(126)

LACTOBACILLUS AS PROBIOTIC

The definition of probiotics was devised in 1974 concurrently with the use of living cultures in feed for various animals in order to replace the application of nutritive antibiotics or chemotherapeutics (135). Until the first concept of probiotics bacteria beginning of the 20 th century when the Ukrainian-born Nobel Prize laureate Elie Metchnikoff (1900) reported that Bulgarians lived longer than other populations and supposed that this was due to their consumption of fermented milk products containing viable bacteria (136). He believed that when the bacillus was consumed, they carried out the fermentation of this product, influencing the microflora of the colon by decease the toxic effects of colonic microflora.

Probiotics are usually defined as live microbial feed supplement that beneficially affects the human and animal by improving its intestinal microbial balance. These bacteria must belong to the natural microflora in order to survive the acid environment of digestive tract system (36-38). They are non-pathogenic bacteria or beneficial bacteria for health and control balance of the gut microflora. Probiotics are the group of organisms known as lactic acid bacteria and are normally consumed in the form of yogurt, fermented milks or other fermented foods (137). Some of the beneficial effect of probiotics consumption include ; they prevent cellular adhesion and invasion of pathogenic bacteria(137), reduce the severity of diarrhea (rotavirus diarrhea, post antibiotic diarrhea) (138), improving intestinal tract health, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, lowering of blood cholesterol (139), decreasing the prevalence of allergy in susceptible individuals (140), risk of certain cancers (140) and they interact and modulate the local and systemic inflammatory immune response (stimulation and regulation) (60, 107, 137, 141) . Lactobacilli are possibly the most commonly studied as a probiotic in people. Reported some strains of *Lactobacillus* considered to beneficial because they are produces vitamin K, lactase, and anti-microbial substances such as bacteriocin, acidolin, acidolphilin and lactocidin.

The recent introduction of the concept of prebiotics has directed attention towards the possibility that alterations in gut microflora induced by the fermentation of non-digestible component of the diet which non-digestible oligosaccharides (NDO), such as the alpha-galactooligosaccharides raffinose and stachyose (142). The term prebiotic was defined as a non-digestible food induced increase in numbers and/or activity predominantly of lactobacilli, bifidobacteria and lactic acid bacteria in the human gastrointestinal tract. In present time, *Lactobacillus* species have been proposed as and are used as probiotic strains (143). Previous study *L. reuteri* and *L. rhamnosus* used as probiotics in man and animal such as in dairy foods (144). The ability of *L. reuteri* to inhibit growth of pathogenic strain and can inhibit effect of TNF-α induced Interleukin-8 (IL-8) by activated epithelium cell. The ability of probiotics to interfere adhesion of *H. pylori* on epithelial cells and their are attenuate *H. pylori*-induced gastritis in human (145).

Mechanism of action of probiotic

The probiotics have been suggested to play a role in a variety of health effects, and mechanism proposed for mediating these effects on human. Lactobacilli belong to the mic ro-organisms m ost f requently us ed t o prepare t he pr obiotics. The m ain of mechanisms of pr obiotic e xert pr otective or t herapeutic effect. M ach w ork r emains t o classify t he m echanisms of pa rticular pr obiotics a gainst m icroorganism pa thogen. T he mechanisms of probiotics against gastrointestinal pathogens addressed in diverse patent applications including production of inhibitory substances such as (oganic acid, hydrogen peroxide, di acetyl, b acteriocins a nd r euteri) a s i ntroduced a bove, m odification of t he environmental a nd c onditions f or gr owth of l actobacilli, c ompetition f or t he nut rients which present the growth substrates an important factor for colonization of lactobacilli, blocking of adhesion sites, degradation of toxin receptor, stimulation and modulation of the immune and non-immune defense mechanisms of the host (146-149).

PATHOPHYSIOLOGY OF *HELICOBACTER PYLORI* AND NSAIDS INDUCED GASTRITIS AND PEPTIC ULCER DISEASE

The r oles of c ytokines i n *H. pylori*-induced g astritis a nd pe ptic ul cer have been investigated. Greater nu mbers of epithelial c ells, intraepithelial, lymphocytes, lamina propria c ells pos itive f or i nterleukin-1β (IL-1β), IL-2, IL-6, I L-8 and t umor ne crosis factor-α (TNF-α) were found in *H. pylori* infected patients. *H. pylori* and NSAIDs are the two major etiologic factors involved in gastritis and peptic ulcer disease. *H. pylori* may induce inflammation is through direct contact with gastric epithelial cells and stimulation of c ytokine r elease (150). The gastric i nflammatory response i nduced b y *H. pylori* consists of ne utrophils, lymphocytes (T a nd B cells), plasma cel ls, and macrophages, along w ith varying de grees of epi thelial cel l de generation a nd i njury. The S everal virulence mechanisms have been proposed for *H. pylori* associated with several clinical outcomes such as the production of urease enzyme, lipopolysaccharide (LPS) and a *cag* pathogenicity i sland (*cag*-PAI) (151). NSAIDs is a commonly cause d yspepsia, a burning, bl oated f eeling i n t he p it of the s tomach. In s ome pe oples, NSAIDs i nduced stomach inflammation (gastritis) or gastric ul cers m ay o ccur. The m ain risk factors for NSAIDs-related pe ptic ulcer complications a re a ge, p ast hi story, us e of hi gher risk individual N SAIDs, dr ug dos e, c oncurrent us e of w arfarin or c orticosteroids. (152)

Production of inflammatory cytokines is stimulated by ulcerogenic factors such as NSAIDs, stress, and *H. pylori* infection which inflammatory cytokines such as IL-1β and TNF-α are cause recurrence of healed ulcer (12, 153). Mehmet N *et. al*. they study the concentrations of pro-inflammatory cytokines such as IL-1β, IL-2R, IL-6, IL-8 and TNF-α in gastric fluid and serum of the patient with grouped according to infection by *H. pylori*. They found that the concentrations of cytokines TNF-α, IL-2R, IL-6, and IL-8 in gastric fluids and serum of *H. pylori*-positive were higher than *H. pylori*-negative control groups (154). The concentration TNF-α was significantly higher in those with active gastritis and neutrophil infiltration into the epithelium than in those with inactive gastritis. The evidence suggested that gastritis is associated with increased gastric mucosal production of TNF-α (14). The biological actions of proinflammatory cytokines are various. The stomach, these multifunctional cytokines are released from monocytes and activated macrophages which modulate several physiological, including gastric acid secretion, somatostatin release, epithelial cell growth, and gastric emptying. Previous studies have examined effects of *H. pylori* infection on gastric acid secretion, usually in duodenal ulcer patients (155). T. SUZUKI *et al*. study proinflammatory cytokines including TNF-α, IL-8 and IL-1 were direct stimulatory effect on gastrin release from isolated G cells (156).

H. pylori is common bacteria causing chronic infection in stomach, and plays an important role in the pathogenesis of gastroduodenal ulceration. *H. pylori* is infects more than 50 % of the human population. They are infection might release of various bacterial and host dependent cytotoxic substances including ammonia, production of a vacuolating cytotoxin and bacterial enzymes which all provide to epithelial damage. However the recruitment and activation of immune cells in the underlying mucosa involves *H. pylori* chemotaxins, epithelial-derived chemotactic peptides (chemokines) such as IL-8, pro-inflammatory cytokines liberated by mononuclear phagocytes (TNF-α, IL-1 and IL-6) as part of non-specific immunity (157, 158). Evidences suggested that eradication of *H pylori* with associated development of the gastritis led to a reduction in the mRNAs encoding both of TNF-α and IL-8 (159).

LACTOBACILLUS AND MODULATION OF IMMUNE

Enteric pathogens may cause gastrointestinal disease in humans and animals, antibiotics have often been used to prevent gastrointestinal disorders. However, the use of antibiotics is no longer recommended due to complications including the emergence of drug-resistant strains. Many reported capability of lactobacilli as probiotic for protect infection by enteric pathogens. The study *Shigella* invades the human intestinal mucosa, thus leading to bacillary dysentery, an acute recto-colitis responsible for lethal complications, mostly in infants and toddler. *S. flexneri* infection leads to the induction of acut inflammation such as IL-8. *L. casei* could attenuate the pro-inflammatory signaling induced by *S. flexneri* after invasion of the epithelial lining (114). *L. casei*, DN-114 001, has been shown to decrease the secretion of TNF-α from the inflammed ileum of Crohn's disease patients. W-H. Lin *et al.* (2005) they study in animal model, *L. acidophilus*, specifically strain LAP5, LAF1 and LAH7, heat-killed and mixed. This heat-killed mix of *L. acidophilus* was used to evaluate the effectiveness of inhibiting *Salmonella typhimurium* invasion into organs (spleen and liver) of BALB/c mice (160).

The human intestinal tract is complex composed of a diverse of bacteria, both pathogenic and nonpathogenic which if imbalance of microbial community is a major causative factor in the pathology of inflammatory bowel disease (IBD). TNF-α is pro-inflammtory cytokine and key in the regulation of many inflammatory disease. Indeed, patients with active Crohn's disease or ulcerative colitis have increased levels of TNF-α. These study is focus on ability of *L. paracasei* was reduced TNF-α expression from derived lipopolysaccharide (LPS) to stimulate the murine macrophages.

Lipopolysaccharide (LPS) is the component of gram negative bacteria cell wall, induce inflammation in gastrointestinal tracts. LPS stimulated TNF-α production and is one of the principal mediators of the inflammatory response in mammals.Probiotic organisms have been used to treat a variety of human intestinal conditions. The recovery of *L. rhamnosus* GG (ATCC 53103) in human biopsies of the patients (161). Evidence investigated by Pena J A and Versalovic J (2003) *Lactobacillus* conditioned media (LCM) of *L. rhamnosus* GG (LGG) can inhibit production of TNF-α by the murine macrophage were activated by LPS of *Escherichia coli* and lipoteichoic acid (LTA) of *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis*. In addition *L.*

rhamnosus GG conditioned media can decreases TNF-α production of *Helicobacter*-conditioned media-activated peritoneal macrophages (58). Similarly Sung O. Kim *et al.* (2006) they study *L. rhamnosus* GG and GR-1, in modulating production of TNF-α in human monocytic cell line (THP-1) and mouse macrophages. They found that *L. rhamnosus* GG and GR-1 were suppress *Escherichia coli*-induced inflammatory cytokine (TNF-α) and induce production of anti-inflammatory cytokines such as IL-10 and Granulocyte colony-stimulating factor (G-CSF) in condition media prepared from live *E. coli*, *L. rhamnosus* GG or *GR-1* exposed mouse bone marrow-derived immortalized macrophages (BMDIM). They suggest that G-CSF secreted from *L. rhamnosus* GG and GR-1 exposed macrophages suppressed TNF-α production induced by *E. coli* or lipopolysaccharide-activated macrophages (50).

Previously study *Lactobacillus* was interfere the adherence of pathogens and inhibit pro-inflammatory cytokines both *in vitro* and *in vivo*. Lactobacilli have been tested in animal model of bowel inflammation. *L. plantarum* strain 299V could attenuate colitis in IL-10-deficient mice induced by specific pathogen-free (SPF) bacteria and evaluated the effect of this probiotic organism on mucosal immune activation (162). Similarly, IL-10-deficient mice were treated with probiotic *L. reuteri* combination with *L. paracasei* and then challenged with *H. hepaticus*. This study found that these lactobacilli diminished inflammation in IL-10-deficient mice infected with *H. hepaticus* (59). The evidences suggest that murine IL-10-deficient mouse colitis model has provided the roles of probiotic *Lactobacillus* spp. as potential prophylactic or treatment in IBD.

Lactobacilli represent components of the commensal mammalian gastrointestinal microbiota and are useful as probiotics. Lactobacilli have been study genotying murine intestinal which *Lactobacillus* isolates were recovered from two groups of mice, colitis mice (IL-10-deficient C57BL/6 mice) and mice without colitis. The study 20 mice without colitis, six *Lactobacillus* species were recovered; the majority of lactobacilli were colonized with *L. reuteri* or *L. murinus* (72% of isolate) and few *L. vaginalis*, *L. johnsonii*, *L. intestinalis*, *L. paracasei*. In contrast, 14 IL-10-deficient mice were colonized with only, *L. johnsonii.* The study immunomodulatory activity with characterization of strains recovered from the mouse intestine. They found that 29

lactobacilli isolated from mice without colitis, 6 isolates (21%) inhibit TNF-α on LPS-stimulated macrophages and none of the 29 lactobacilli recovered from colitis mice (32).

The mechanism of the evident anti-inflammatory action of probiotic organisms is unclear. Several evidences suggest that TNF-α is pro inflammatory cytokine. Recent have been reported the (10 ng/ml) TNF-α was induced IL-8 production in human colon epithelial cell lines. This study live *L. reuteri* as concentration 1 x 10^7cells/ml significantly inhibited TNF-α induced IL-8 secretion, similarly *L. reuteri* inhibits IL-8 release induced by *S. enterica* serovar Typhimurium. Furthermore, the effect was not reproduced by conditioned media, heat-killed or gamma-irradiated organisms. They suggest that live but not heat-killed or gamma-irradiated *L. reuteri* dose dependently inhibited constitutive synthesis by T84 and HT29 cells of IL-8 and that induced by TNF-α (163). Similarly, *L. bulgaricus* was suppress IL-8 secretion in intestinal epithelial cell (HT29) when stimulated by proinflammatory cytokines TNF-α and decreased expression of nuclear NF-kB p65 activation in intestinal epithelial cell in the experiment (164) likely *L. rhamnosus* GG (LGG) are capable of downregulating TNF-α induced IL-8 production in caco-2 cells (165).

The mechanisms of probiotics are several include development of epithelial barrier function and immunoregulatory effects (166). The determined *L. plantarum* can modify the corrosive effects of TNF- α on intestinal epithelial cells. They study Caco-2 cells were incubated with TNF- α alone or in the presence of *L. plantarum*, measured epithelial barrier and IL-8 secretion was measured using an ELISA method. The result *L. plantatum* decrease in epithelial barrier and TNF-α-induced IL-8 secretion was reduced by *L. plantarum* (167).

In present study *H. pylori* strain SS1 was colonizes in C57BL/6 mouse and leads to the development of associated gastritis in the lamina propria and the levels of proinflammatory chemokines macrophage inflammatory protein 2 (MIP-2) and keratinocyte-derived cytokine (KC) in the serum and gastric tissue. This study *L. johnsonii* La1 at 12 weeks only mice maintained significantly mild chronic gastritis, MIP-2 serum levels were reduced during the early stages and did not suppressive effect on *H. pylori* colonizing numbers and other lactobacilli, such as *L. amylovorus* DCE 471 and *L. acidophilus* IBB801, did not reduce *H. pylori*-associated gastritis. These

observations suggest that *L. johnsonii* La1 can attenuate *H. pylori*-induced gastritis *in vivo* during the early infection stages, which reducing proinflammatory chemotactic signals responsible for the recruitment of neutrophils and in the lymphocytes lamina propria (168).

H. pylori infection was associated with gastroduodenal disease which the treatment not always successful in eradicating the bacterium and may be have side effects. The study *L. salivarius* was efficiently eradicated *H. pylori* in gnotobiotic murine model (63). Recently, the yogurt containing of *L. gasseri* OLL2716 improve *H. pylori* infection-induced gastric mucosal imflammation. The investigated of *L. gasseri* OLL2716 (LG21) exibites a gastroprotective action against of acut gastric lesion or antral ulcer in rats. Acut gastric lesion was induced by oral administration of 0.6 M HCL. *L. gasseri* OLL2716 (LG21) yogurt dose-dependently was inhibited acut gastric lesion and antral ulcer (64). Study in human trials have been reported *L. gasseri* OLL2716 (LG21) strain by use yogurt containing LG21 twice daily for a further 8 weeks in human with *H. pylori* infected. They found that the effective in both suppressing *H. pylori* colonization and reducing gastric mucosal inflammation in humans (65, 66). IL-8 is chemokine which a potent neutrophil chemoattractant and activating agent, accumulating evidence indicates that IL-8 plays a major role in the mucosal inflammation cauased by *H. pylori* infection (67, 68). Akira Tamura *et al*. (2006) they are study *L.gasseri* OLL2716 (LG21) suppress *H.pylori*-induce IL-8 production in human gastric epithelial cell line (MKN45). In contrast, the UV- or heat-treated LG21 could not suppress *H. pylori*-induced IL-8 production in *vitro*. TNF-α is well-known to induce IL-8 production in gastric epithelial cells. Indeed, in the present study 10^8 CFU/mL LG21 inhibited the TNF-α-stimulated IL-8 production. Furthermore they study LG21 can inhibit the adhesion of *H. pylori* to host cells which associated with dose dependent of LG21. Finally, they found that live LG21 were found to suppress *H. pylori*-induced IL-8 production in within gastric mucosa of patients (66).

METHOD FOR IDENTIFICATION OF GENUS *LACTOBACILLUS*

1. Phenotypic method

The species of *Lactobacillus* may be difficult to identify by conventional biochemical methods, although simplified approaches are useful for presumptively assigning organisms to this genus. Isolates were identified based on Gram stain, catalase test, and fermentation patterns using API 50 CH kits (169). *Lactobacillus* organisms are generally gram stain morphology, catalase negative, oxidase negative, vancomycin resistant.

Gram stain morphology

Gram staining is based on the ability of bacteria cell wall to retaining the crystal violet dye during solvent treatment. The cell walls for gram-positive bacteria have a higher peptidoglycan and lower lipid content than gram-negative bacteria. It is almost always the first test performed for the identification of bacteria. These microorganisms that are stained by the gram's method are commonly classified as gram-positive or gram-negative under microscopic examination. *Lactobacillus* is gram-positive bacteria (170).

Catalase test

Catalase is enzyme found in all living organisms. The testing of catalase enzyme was used to differentiate between bacterial species in laboratory. The test is placing a drop of hydrogen peroxide on a slide, picked bacterial colony and smear into the hydrogen peroxide drop. The result show that catalase-positive by bubbles or froth forms and catalase-negative without bubbles from. This test is particularly for distinguishing staphylococci and micrococci which are catalase-positive, but lactobacilli, streptococci and enterococci which are catalase-negative. Which catalase test alone cannot identify a particular organism, combined with other tests for indentify lactobacilli from streptococci and enterococci. The functions of catalase enzyme include catalyzing in the decomposition hydrogen peroxide into water and oxygen.

Vancomycin susceptibility test

Lactobacillus is usually sensitive with vancomycin. In a previous study *Lactobacillus* identification with vancomycin susceptibility test forty strains of *Lactobacillus* isolated from probiotics supplement or functional food, found that all *L. acidophillus* and *L. delbleuckeii* were sensitive with vancomycin ,while *L. rhamnosus* was resistant (171). Pena *et al*. (2004) lactobacilli can be divided into vancomycin-resistant such as *L. murinus*, *L. reuteri*, *L. vaginalis* and vancomycin- susceptible groups such as *L. acidophilus* group and *L. casei* group (32).

API 50 CHL

API 50 CHL is carbohydrate fermentation which the principle of fermentation carbohydrate for identification strains of *Lactobacillus* to the species level. API 50 CHL is used for identification of *Lactobacillus* by fermentation is revealed by a change of color in the tube. The color change by organism can product of acid and was detected indicator in medium (106). Previous study *L. acidophilus* complex which cannot be distinguished biochemically has been subdivided into six distinct species; *L. acidophilus, L. crispatus, L. gasseri, L. gallinarum, L. amylivorus* and *L. johnsonii* so the studies genotypic method as important for identification species of lactobacilli.

2. Genotypic method

The taxonomy of lactobacilli has expanded as a result of genomic sequence analysis. DNA sequencing of informative target regions, such as the 16S rRNA gene and the 16S-23S ribosomal DNA intergenic spacer region (ISR), has resulted in useful for definitive species identification within *Lactobacillus* species complexes. The rapid developments of genus-, species-, and strain specific 16 SrRNA nucleotide have successful. DNA sequencing is the gold standard for identifying the products of amplification reactions was generally performed by polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) method

The polymerase chain reaction (PCR) is a technique widely used in molecular biology which technique for amplification *in vitro* by the simultaneous primer extention of complementary stands of deoxynucleotide-tri-phosphate (DNA). The PCR was amplified a piece of specific DNA regions (DNA template). DNA polymerase carries out the synthesis of a complemantary strand of DNA in the 5' to 3' direction using single strand DNA template but starting from double-stranded region. The basic of PCR set up requires several reagents and components are including DNA template, oligonucleotides primer which are complementary to DNA region, DNA polymerase (172), deoxynucleoside triphosphates (dNTP), buffer solution for optimum activity and stability of the DNA polymerase, magnesium (Mg2+) and potassium ions. The cycling reactions are repeated for 30 or 40 cycles. This is used on an automated cycler, which the tubes with the reaction mixture can heat and cool in a very short time.

The step of PCR as shown in Figure 6

Denaturation initiation is heating the reaction to a temperature of 94-96 °C, for 5-10 minutes because DNA polymerases that require heat activation. Denaturation is consists of heating the reaction to 94-98°C for 20-30 seconds which melting of DNA template by disrupting the hydrogen bonds between complementary bases of the DNA strands into single stranded DNA. Annealing is lowered to 50-65°C for 1 minutes allowing annealing of the primers to the single-stranded DNA template. Extension or elongation, is consists of heating the reaction to 72-80°C which is depend on DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTP that are complementary to the template in 5' to 3' direction. Final extention, this single step is occasionally performed at a temperature of 70-74°C for 5-15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended and hold at 4°C for an in definite time before PCR product detection.

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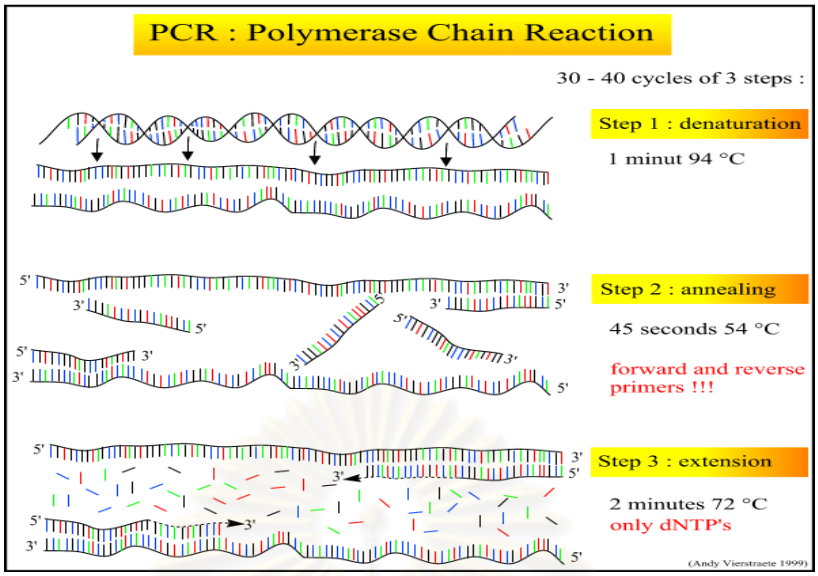


Figure 6. Schematic diagram of steps in PCR.

DNA sequencing (Dideoxy sequcing)

The Sanger method has served as the cornerstone for genome sequence production since 1977, close to almost 30 years of tremendous utility (173). The dideoxy enzymatic method as originally developed by Frederick Sanger utilizes *E.coli* DNA polymerase I to synthesize a complementary copy of single-stranded DNA template (174). This technique uses sequence-specific termination of a DNA synthesis by using modified nucleotide substrates. In chain terminator sequencing, extension is initiated at a specific site on the template DNA by using oligonucleotide primer complementary to the template region. The oligonucleotide primer is extended using a DNA polymerase an enzyme that replicates DNA.

The Sanger sequencing method capitalizes on the ability of DNA polymerase I of *E.coli* Atkinson *et al.* showed that the inhibitory activity of 2',3'-dideoxynucleotide triphosphate (ddDTP) on DNA polymerase I depends on its being incorporated into the growing oligonucleotide chain in the place of ddDTP. Because the ddDTP contains no 3'-hydroxyl group, the chain cannot be extended, chain elongation is terminated selectively at A, T, C or G occurs specifically at positions (175).

The conceptually most satisfying approach to the introduction of a label during the Sanger sequencing protocol is via a labelled terminating dNTPs. Using such fluorescent analogues bypasses the difficulties of labeling the primer or of designing the primer for a successful enzymatic internal labelling. The 5' terminus of the primer has been a popular target for the attachment of a variety of labels such as radioactive isotopes, fluorescent dyes or other tags are used (173) (Figure 7).

The Sanger sequencing reaction mixture including DNA template, thermostable DNA polymerase, a primer, dNTPs (dATP, dGTP, dCTP and dTTP) and ddNTPs (ddATP, ddGTP, ddCTP, or ddTTP) fluorescently labeled nucleotides, which ddNTPs are the chain-terminating nucleotides, lacking a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides during DNA strand elongation. The condition of DNA sequencing is similar with PCR condition using a commercially available themal cycling machine.

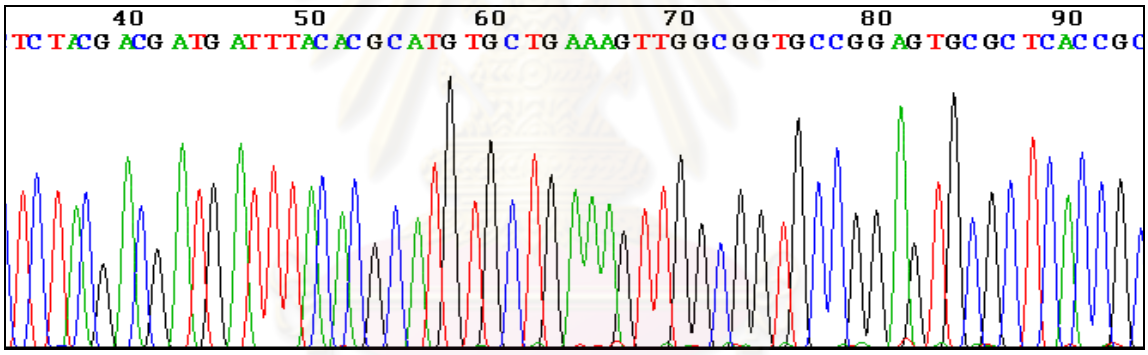


Figure 7. Chromatogram of sequencing by automate sequencer

Genotyping of *Lactobcillus*

Indigenous *Lactoacillus* believed to play important role in control the gastrointestinal tracts and maintenance its normal state. The identification of lactobacilli based on physiological and biochemical criteria is very difficult and uncertain (29). Furthermore, non cultivable organisms and organisms with ambiguous biochemical profiles can be identified. Recently, have the reported on the application of 16S rRNA gene sequencing for bacterial identification. The studies 16S rRNA or 23S rRNA-targeted hybridization probes and polymerase chain reaction (PCR) primers have been applied successfully to the detection and identification of some *Lactobacillus* species.

Analysis of bacteria based on 16S rRNA primers is a sensitive and specific technique to identify gastrointestinal tracts that are difficult to cultivate.

The several studies design and validate primer for detection of lactobacilli in gastrointestinal tracts colonization. Rekha, R. *et al*. (2006) they study design and validation of genus-specific primers for human gut flora using polymerase chain reaction (PCR). They were designed six different primers sets to differentiate following of anaerobic genera *Bifidobacterium* (Bif), *Ruminococcus* (Rum), *Lactobacillus* (Lacb), *Campylobacter* (Camp), *Peptococcus* (Pep), and *Clostridium* (Clos) which each primers used for the PCR reaction showed specificity for targeted genera (176). John Penders *et al*. (2006) they determine the gut microflora composition in early infancy which was detected by quantitative real-time polymerase chain reaction assays. The primers were specific for lactobacilli, bifidobacteria, *Escherichia coli*, *Clostridium difficile*, *Bacteroides fragilis* group and total bacterial counts (177). Similarly in 2007 Jinjin Chen and group they studies was quantify lactobacilli and bifidobacteria in healthy breast-fed neonates. These study show that lactobacilli and bifidobacteria colonization in the gut. The genus specific 16S rRNA-tageted primer sets, which *Lactobacillus* primers L159-f and L677-r have been validated by Heillig *et al*. As shown in Table 6 (178, 179).

Pena *et al.* (2004) they study the genotyping of lactobacilli isolates from mouse intestine by 16S rDNA sequencing which PCR was amplified with primers 16S-8F and 16S-1541R (universal primer) and 16S rDNA gene was sequenced by using an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (32). Roos S, Engstrand L and Jonsson H (2005) they study the composition of *Lactobacillus* flora from human gastric mucosa and study genetic characteristics by complete of 16S rRNA gene sequences for the strains were amplified by PCR with bacteria-specific primer. These study analysis 16S rRNA gene of lactobacilli 15 isolates and were divided into four groups which those of all members of *L. reuteri* subgroup and *L. delbrueckii* subgroup (35). The discovery of PCR and DNA sequencing, the 16S rRNA gene is highly conserved within a species and among species of the same genus and can be used as the new “gold standard” for decision of the species of bacteria (180).

Table 6. Target groups and sequences of the PCR primers in previous study

Target Organism	Name primer	Primer Sequence (5' to 3')	PCR Product	Annealing Temp.	References
Genus *Lactobacillus*	Lacb (F)	TGCCTAATACATGCAAGTCGA	318 bp	52.0	(176)
	Lacb (R)	GTTTGGGCCGTGTCTCAGT			
Lactobacillus spp.	F primer	AGCAGTAGGGAATCTTCCA	341 bp	59.0	(115, 177)
	R primer	CACCGCTACACATGGAG			
Lactobacilli	L159-f	GGAAACAG(A/G)TGCTAATACCG	519 bp		(178, 179)
	L677-r	CAC CGC TAC ACA TGG AG			
Universal primer	16S-8(F)	AGAGTTTGATCYTGGYTYAG	1,500 bp	57.0	(32)
	16S-1541(R)	AAGGAGGTGWTCCARCC			

However, some time in lactobacilli as closely related species which 16S rRNA primers have not been used caused very little sequence variation observed between the 16s rRNA genes of closely related microorganisms (181). The sequence of the 16S-23S rRNA intergenic spacer region (ISR) were shown greater variations than that of the 16S rRNA structural gene for designing specific primers to identify closely related species. Yu-Li Song *et al*. (2000) study lactobacilli isolated from Japanese stool specimens by two-step multiplex polymerase chain reaction (PCR) assays. The first step of multiplex PCR was used for grouping of lactobacilli with a mixture of group-specific primers followed second step with four sorts of species-specific primer mixtures for identification at the species level. The primers were designed from nucleotide sequences of the 16S-23S rRNA intergenic spacer region (ISR) and its flanking 23S rRNA gene of genus

Lactobacillus. The phylogenetic tree were differentiated into four groups of lactobacilli (182). The several reports on specific PCR identification for lactobacilli, mainly based on ribosomal genes and the ribosomal intergenic region (183, 184). Previous systems are not sensitive enough to differentiate bacteria under the species level which, have been molecular typing methods such as the random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism, pulsed-field gel electrophoresis and ribotyping as technique have been used for discrimination of *Lactobacillus* strains.

LIPOPOLYSACCHARIDES (LPS)

Lipopolysaccharide is the major components of the outer membrane of gram negative bacteria. LPS is localized in the outer layer of the membrane and protect cell against the action of bile salts, lipophilic antibiotics, phagocytosis and cell lysis. Lipopolysaccharides (LPS, endotoxin) represent a major virulence factor of gram-negative bacteria, which can cause septic shock in mammals. The molecule stucture of LPS is consists of three distinct regions: the highly hydrophobic lipid A (or endotoxin), hydrophilic core oligosaccharide is nonrepeating of oligosaccharide and repeating unit of oligosaccharide or O-polysaccharide (O-antigen) as shown in Figure 8 (185, 186).

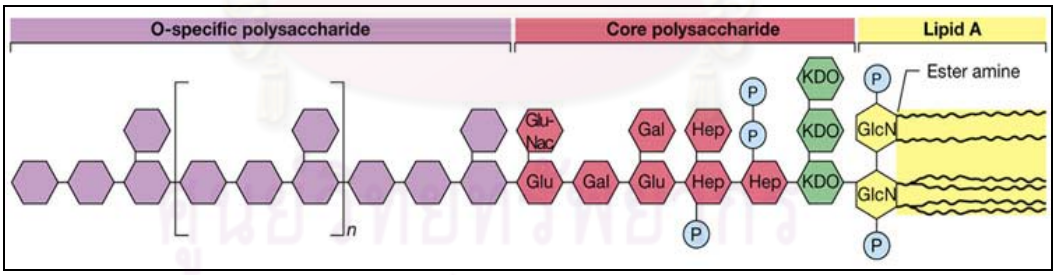


Figure 8. Chemical structure of LPS (endotoxin), (Hep) L-glycerol-D-manno-heptose; (Gal) galactose; (Glc) glucose; (KDO) 2-keto-3-deoxyoctonic acid; (NGa) N-acetyl-galactosamine; (NGc) N-acetyl-glucosamine.

The most of O-specific polysaccharide chain are repeating units of oligosaccharides which display a structural diversity of each strain. The sugar elements determine the serological O specificity of particular strains. Since lipopolysaccharides confer antigenic properties on the cell, they have main antigen intermed O antigens.

Lipid portion or Lipid A is identical among all gram negative species. Recent genomic data have simplify study of LPS assembly in diverse gram-negative bacteria which highly toxic and motion a powerful immune response. Bacteria have been common serotypes of surface antigens (group O, group H) which examples *E. coli* O127:B8.

Lipopolysaccharide-mediated signal transduction

LPS is recognized by cells of the innate immune system. LPS is an incredibly potent initiator of immune cascades. In conditions where the body is exposed to LPS excessively or systemically such as LPS enter the blood stream, a systemic inflammatory reaction can occur, leading to multiple pathophysiological effects, such as endotoxin shock, tissue injury, and death. However, endotoxin does not activate directly against cell or organs but through activation of the immune system, which especially through monocytes and macrophages, with the release of a range of pro-inflammatory mediators, such as tumor necrosis factor (TNF-α) (185). The biological response to LPS is mediated by a receptor complex Toll-like receptor 4 (TLR) 4, CD14, and LBP. TLR4 is a family of innate immunity receptors that possess a large extracellular domain of leucine-rich repeats, a single *trans*-membrane segment, and a smaller cytoplasmic signaling region that engages the adaptor protein MyD88 when stimulated by antigen, this receptor initiates an intracellular signaling cascade that results in the activation of Erk, Jnk, p38, Akt, and NF-kB, AP-1 binding size which, as shown in Figure 9 (187).

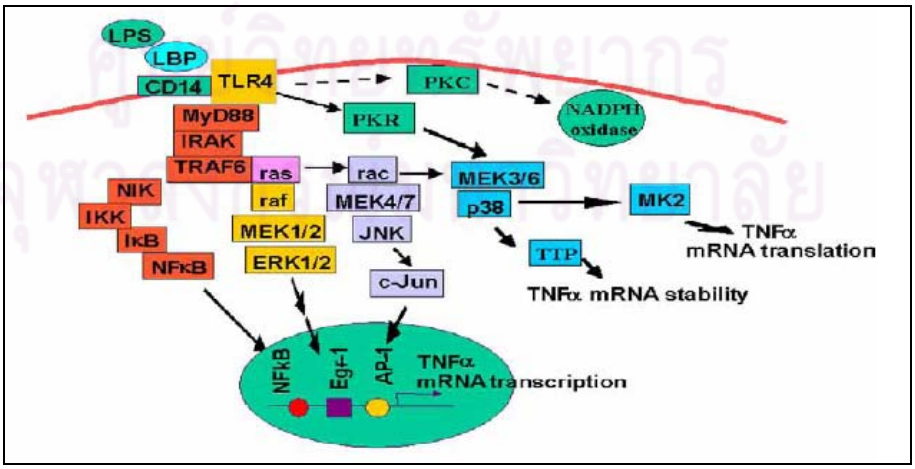


Figure 9. Lipopolysaccharide stimulated signal which increase TNF-alpha production in macrophages (187)

TUMOR NECROSIS FACTOR-ALPHA (TNF- α)

Tumor Necrosis Factor-alpha (TNF-α) is a member of a family of cytokines. TNF-α is a highly pleiotropic cytokine that plays a central role in inflammation and apoptosis (19). TNF-α is a major mediator of inflammation as well as apoptosis and immunity, and it has been associated in the pathogenesis of a wide spectrum of human diseases, including sepsis, diabetes, cancer, osteoporosis, allograft rejection and autoimmune diseases such as multiple sclerosis (20), rheumatoid arthritis, and inflammatory bowel diseases (21, 22). TNF-α is beneficial in activating the innate immune response, inappropriate production of TNF-α leads to inflammation, tissue destruction, and organ injury. TNF-α is produced by many different cell types, but especially by macrophage. The main sources *in vivo* are stimulated monocytes, neutrophil, and endothelial cells (25). The TNF-α was produced by macrophages, T-cells and B-lymphocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glial cells and keratinocytes after stimulation. TNF-α is reported to promote inflammatory cell infiltration by upregulating leukocyte adhesion molecules on endothelial cells, serve as a chemotactic agent for monocytes (188), and activate phagocyte killing mechanisms. Additional beneficial functions of TNF-α include its role in the immune response to bacterial, and certain fungal, viral, and parasitic invasions (188) as well as its role in the necrosis of specific tumors. TNF-α cytokine family induce hepatic expression of acute phase proteins and increases vascular permeability, by which recruiting macrophage and neutrophils to a site of infection. The deficiencies in either TNF-α or its receptors can increase susceptibility to infection by intracellular pathogens.

Monocytes and macrophages are major cellular components of the innate of the immune system which ability to produce TNF-α, in response to bacteria and bacterial fragments, such as lipopolysaccharide (LPS) (24). TNF-α is a key mediator in a host response to infections, LPS is (endotoxin), a constituent of the outer membrane of gram-negative bacteria, can initiate a cascade of inflammatory mediators that can lead to systemic inflammation (25). LPS is an especially potent stimulus for TNF-α by LPS stimulated macrophages. Previous investigated the effects of LPS on the expression of cytokines secreted by bovine polymorphonuclear neutrophil leukocytes (PMN), they detected the expression of TNF-α by ELISA (26). Lipoteichoic acid (LTA) and lipopolysaccharide (LPS), the toxicants from bacteria, are potent inducers of

inflammatory cytokines, such as tumor necrosis factor-alpha (TNF) in macrophages notably, increasing evidence suggests that macrophages also play an important role in the development of the low-grade inflammation (27).

General of TNF-α biology

TNF-α is a soluble cytokine has been identified that was produced upon activation by the immune system. In 1984, the cDNA of TNF-α was cloned, the structural and functional homology to lymphotoxin (LT) was realized, and two membrane receptors, each capable of binding both cytokines, were identified (189). TNF-α gene is located on chromosome 6 in man and chromosome 17 in the mouse (190, 191). TNF-α has a subunit molecular mass of 17 kDa of protomers are composed of two antiparallel β-pleated sheets with antiparallel β-strands forming a 'jelly roll' structure, typical for the TNF ligand family. TNF is primarily produced as a 212 amino acid-long type II transmembrane protein arranged in stable homotrimers (188, 192). Monocytes express at least five different molecular forms of TNF-α with molecular masses of 21.5-28 kDa. They mainly differ by post-translational alterations such as glycosylation and phosphorylation. Biological action of TNF-α is bind to two detached cell surface receptors, TNF receptor1 (TNFR1) (55 kDa) and TNFR2 (75 kDa). TNF-α is polypeptide cytokine were produced during infection, injury, or invasion, has proved its triggering the lethal effects of septic shock syndrome, cachexia, and other systemic manifestations of disease and TNF-α is a proinflammatory cytokine known to play a key role in the pathogenesis of IBD (193).

Detection of TNF-α

Enzyme Linked Immuno-Sorbent Assay (ELISA) method is biochemical technique used in immunology to detection and quantitation of the presence biological substances such as antibodies, proteins, peptides, hormones and cytokine. The ELISA is use combine the specificity of antibodies with the sensitivity by simple enzyme assay and can detect and measure concentration of antigen or antibody with quick. ELISA method can provide a specific, sensitive and rapid method for detection of TNF-α in the serum of patient and it is important that the assay used should be sufficiently sensitive to detect low levels of TNF-α (194).

Sandwich enzyme-linked immunosorbent assay (Sandwith ELISA)

In 1990 Adolf GR and Lamche H R they were developed a rapid, simple and highly sensitive sandwich enzyme immunoassay (ELISA) for the detection and quantification of human tumor necrosis factor (TNF-α) in serum (195). The Sandwich ELISA is method as measures the amount of antigen sample by use two layers of capture and detection antibody. Sandwich ELISA is method for quantitation of antigens in the sample when the concentration of antigens is low or in high concentrations of contaminating protein. An immunoassay for TNF-α is described using a sandwich system which employs a mouse monoclonal as the capture antibody and a polyclonal rabbit anti-TNF-α as the detection antibody (196).

General procedure of sandwich ELISA are prepare capture antibodies purified and bound to a solid phase attached to the bottom of a plate well, block non specific binding site, antigen sample contain to the plate, detection antibodies that to bind with specific antigen, remove the unbound antigen by wash, add the secondary antibody conjugated to an enzyme which specific with detection antibodies, wash that unbound of antibody-enzyme conjugates, add substrate which is converted into color fluorescent or electrochemical by enzyme and measure the absorbance of fluorescence or electrochemical signal of the plate wells to determine the presence and quantity of antigen as shown in Figure 10.

The advantages sandwich ELISA for the detection of antibodies are including the convenience of the microtiter plate for testing large numbers of samples, the absence of radioactive tracers and precipitation steps, the high stability of the reagents thus, the possibility of testing samples from various species without modification of the assay and the ability to detect low-affinity antibodies which the absence of competitive reactions (195).

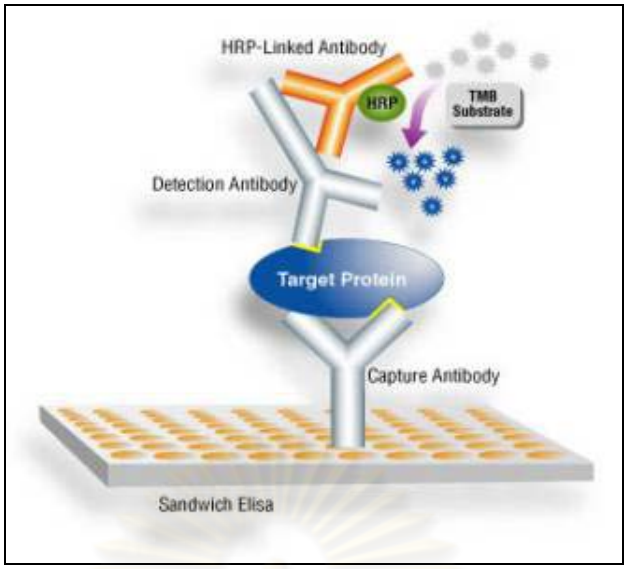


Figure 10. Sandwich enzyme-linked immunosorbent assay

HUMAN ACUTE MONOCYTIC LEUKEMIA CELLS (THP-1)

THP-1 cells are acute monocytic leukemia cells (ATCC TIB202). The source of THP-1 cells derived from the peripheral blood of a 1 year old male with acute monocytic leukaemia. This cell line had differentiation, lysozyme synthesis, phagocytosis, Fc receptor, IL-1 production, complement (C3b) receptors and express HLA A2, A9, B5, DRw1, and DRw2 antigens and lack surface and cytoplasmic immunoglobulin (197). The growth properies of THP-1 cells are suspension cells with monocytoid morphology and morphology of THP-1 cell is large, round, single cells. Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) (198) which matures into macrophage-like adherent cells following stimulation with phorbol 12-myristate 13-acetate or 1 α, 25-dihydroxy vitamin D3. The monocytic cell line was characterized by the presence of alpha-naphthyl butyrate esterase activities which could be inhibited by NaF, lysozyme production, the phagocytosis of latex particles and sensitized sheep erythrocytes.

THP-1 cells are growth in suspension RPMI 1640 plus 10% fetal bovine serum (FBS), 2mM L-Glutamine and maintain culture between 2-9x10^5cells/ml. 37°C, 5% CO_2. THP-1 cell is origin from human and no evidence for the presence of infectious viruses or toxic products. However, it is recommended that cultures are handled under Biosafety Level 2 containment (198).

CHAPTER IV

MATERIAL AND METHODS

1. Patients and clinical specimens

A total of 272 patients presenting with dyspepsia were enrolled in the study. There were 98 (36.03%) males and 174 (63.97%) females. The mean age was 49±15 years (range 18-80 years). These patients were out patients at Gastrointestinal unit, King Chulalongkorn Memorial hospital during 12 months peroid from October 2006 to October 2007. They were divided into three groups by endoscopic findings as follows: group 1, 70 patients with mild gastritis (mean age 46±16; range 19 to 78 years; 23 males; 47 females); group 2, 158 patients with severe gastritis (mean age 49±15; range 18 to 76 years; 48 males; 110 females) and group 3, 44 patients with peptic ulcer (mean age 59±13; range 31 to 79 years; 27 males; 17 females). All patients gave informed consent. Exclusion critetia included the patients who had bleeding in the stomach, cirrhosis, tuberculosis and AIDS. This study was approved by the Ethics Committee for Human Research of Faculty of Medicine, Chulalongkorn University.

All patients enrolled in the study underwent upper esophagogastroduodenal endoscopy (EGD). Before endoscopy, a throat swab was made by using sterile cotton swab and inserted into 2 ml of de Man-Rogasa-Sharpe (MRS) broth (Oxoid, England). One biopsy sample of each patient taken from the antrum (about 0.3 mm in size; Figure 11) by using a disinfected endoscope was placed in 200 µl MRS broth. The specimens were processed immediately upon receipt.

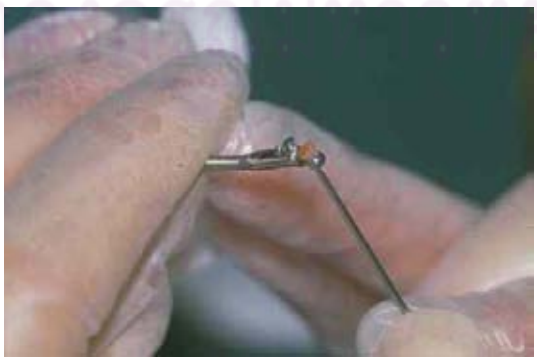


Figure 11. The collection of gastric biopsy sample.

2. Culture of peptic biopsy samples and throat swabs

Gastric biopsy samples in MRS broth were treated in an ultrasonic water bath (GEN-PROBE, Geprufte & Sicherheit, Germany) for 2 min to separate the bacterial cells from the biopsy into MRS broth. This treatment has shown to be optimal for separation of bacterial cells from the stomach biopsies without damaging them (199). The gastric biopsy suspension of 100 µl was spreaded on MRS agar in duplicate. The culture plates were incubated anaerobically (10% CO_2, 10% H_2, and 80% N_2) at 37°C for 48-72 h in anaerobic chamber (Concept Plus, Ruskinn technology) or anaerobic jar (Oxoid, England).

Throat swabs were shaken gently for 5 times and 20 µl of the suspension was streaked on MRS agar in duplicate and incubated anaerobically at 37°C in anaerobic chamber (Concept Plus, Ruskinn Technology, England) or anaerobic jar (Oxoid, England) for 48-72 h. Bacterial colonies that developed on MRS agar with different appearance were picked and each was streaked on a new MRS agar. After an aerobic incubation, single pure colony was isolated and subculture for experimental use.

3. Selection of *Lactobacillus* isolates

Each of the isolate was examined by gram stain. Isolates that were gram-positive rod or coccobacilli were tested for catalase by placing a drop of 3% hydrogen peroxide (H_20_2) solution on the cells. Immediate formation of bubbles indicated the presence of catalase in the cell. Subsequently, only the isolates which were gram-positive rod and catalase-negative were tested for vancomycin (VA) susceptibility test as described by Pena *et al*. (32), Briefly, *Lactobacillus* isolates were suspended in 0.85% normal saline solution (NSS) to 0.5 McFarland standard and swabed onto MRS agar plates. Vancomycin imprenated disks (VA 5 µg/disc, Oxoid, England) were applied to bacterial cultures, which then grown in anaerobic condition at 37°C for 24-48 h. The isolate displaying inhibition zone of greater than 15 mm was considered susceptible. *Lactobacillus* isolates, differing in colony appearance or cell morphology, were selected from bacterial cultures of each patient. One to seven colonies were picked from the culture with similar colony appearance. All isolates which were gram-positive, regular rods or short rod or coccobacilli and catalase- negative were maintained as frozen

cultures in MRS broth with 20% (v/v) sterile glycerol (Oxoid, England) and stored at -80°C (Sanyo, Japan) for experimental use.

4. Genotypic characterization of *Lactobacillus* Isolates

4.1 DNA preparation

DNA was extracted using High Pure PCR Template Preparation Kit (Roche, USA) following the manufacturer's instructions. A loopful of pure culture was suspeneded in 200 µl of double-distilled water (DDW) in 1.5 ml microcentrifuge tube into density of 10^9cell/ml, centrifuged at 3,000xg for 5 min and re-suspended in DNAse- and RNAse-free distilled water (Gibco; Invitrogen, UK.). The berterial cells were lysed by adding 15 µl lysozyme solution (10mg/ml Tris-HCL, pH 8.0) (Ameresco, UK) and incubated at 37°C for 15 min. After incubation, the samples were added 200 µl of Binding Buffer and 40 µl Proteinase K (Roche, USA), mixed immediately and incubated at 70°C for 10 min. After incubation, 100 µl of Isopropanol (MERCK, Germany) was added and mixed well. The liquid sample was then transfered to High Filter Tube in one Collection Tube and centrifuged at 8,000 x*g* for 1 min. After centrifugation, the Filter Tube was removed from the Collection Tube and combined with a new Collection Tube. Five hundred microliters of Inhibitor Removal Buffer was added to the upper reservoir of the Filter Tube and centrifuge at 8,000x*g* for 1 min. After centrifugation, the Filter Tube was removed from the Collection Tube and combined with a new Collection Tube. Five hundred microliters of wash Buffer were added to the upper reservoir of the Filter Tube and centrifuged at 8,000 x*g* for 1 min and the flowthrough was discarded. The Filter Tube-Collection Tube assembly was centrifuged at maximum speed (aproximately 13,000xg) for 30 s for removal of the residual buffer. After centrifugation, the Collection Tube was discarded and inserted into a clean, sterile 1.5 ml microcentrifuge tube. Two hundred microliters of Elution Buffer was prewarmed at 70°C and added into the upper reservoir of the Filter Tube and centrifuged at 8,000x*g* for 1 min. The eluted DNA was stored at 4°C or frozen at -20°C for later analysis.

4.2 DNA amplification by polymerase chain reaction (PCR)

4.2.1 *Lactobacillus* group-specific primers

PCR was performed by using primers designed according to the sequence of the genus *Lactobacillus* on 16S r RNA gene as described by Penders *et al* (177). The amplication product was 341 bp. The reaction was performed in 0.5 ml PCR tube. Amplification was performed in 50 µl mixture containing 5.0 µl of 10X buffer (10 mM Tris-HCl, 50 m M KCl), 2.5 m M of $MgCl_2$, 0.4 m M of deoxynucleoside triphosphate (dNTPs; dATP,dCTP,dGTP,dTTP), 10 pmoles of each primer. PCR was amplified using forward primer L341-F (5'-AGC AGT AGG GAA TCT TCC A-3') and reverse primer L341-R (5'-CAC CGC TAC ACA TGG AG-') (Invitrogen Custom Primers, Hong Kong), 1.25 U Fast start Taq DNA polymerase (Roche, Germany), 2.0 µl of the DNA template, and DNAse- and RNAse-free distilled water (Gibco-Invitrogen, UK.) in a volume of 50 µl. Amplification of 16S rRNA gene was performed under the following PCR condition: denaturation at 95°C for 5 min, followed by 35 cycles of denaturation 95°C for 30 s, primer annealing at 57°C for 1 min, extension at 72°C for 1 min and one cycle of 72°C for 5 min with Eppendorf Master Cycler gradient PCR system (Thermal Master cycler gradient, Germany).

4.2.2 Universal primer

Genotypic characterisation by PCR followed with sequencing of 16S rRNA gene was performed by the method of Pena *et al* (32). Universal primers were designed according to the sequence of the 16SrRNA gene of the microorganism. The amplication product of 16S rRNA gene was about 1,500 bp. The reaction was performed in 0.5 ml PCR tube. Amplification was performed in 50 µl mixture containing 25µl of (2X) Hot start master mix (GE Healthcare illustra, UK) which contained 10 mM Tris-HCl, 50 mM KCl, 3 m M MgCl2, 0. 4 m M deoxynucleoside triphosphate (dNTP), 2 U Taq DNA polymerase, Hot Start Activator protein and Stabilizers and 50 pmoles of each primer. The primer 16S-8F (5'-AGA GTT TGA TCY TGG YTY AG-3') and 16S-1541R (5'-AAG GAG GTG WTC CAR CC-3') (Invitrogen Custom Primers, Hong Kong), 2.0 µl of the DNA template/sample, and PCR water (Roche Applied Science) to a volume of 50 µl. The PCR cycling parameters were initial DNA denaturation at 95°C for 5 min,

followed by 35 cycles of denaturation 95°C for 30 s, primer annealing at 57°C for 1 min, extension at 72°C for 1 min and one cycle of 72°C for 5 min with Eppendoft Master Cycler gradient PCR system.

4.3 Detection of amplication product

Ten microliters of PCR product were mixed with 3 µl of gel loading dye (20% ficoll, 0.05% bromophenol blue) and analyzed in electrophoresis apparatus (Wealtec, Taiwan) on 1.0% Ultrapure™ Agarose (Reseach, USA) gel, consisted of 1% solution of ethidium bromide (50 µg/ml) in 0.5X Tris-Boric Acid-EDTA (0.5X TBE) buffer pH 8.0. The electrophoresis was carried out at 100 volts for 60 min. A molecular ladder of 100-bp plus was used to estimate the size of PCR fragments. Gels was visualized by UV transillumination (Bio-Rad) and recorded by Cemera Gel Doc^{TM}MZL (Bio-RAD, USA).

4.4 Sequencing of the 16S rRNA gene

The sequencing of the 16S rRNA gene was performed according to the method as previously described (32). PCR products (approximately 1,500 bp) were purified by using QIAquick PCR purification kit or QIAquick gel extraction kit (Qiagen Inc., USA) Sequencing was performed by using 10 ng purified PCR product. Sequencing was performed using the same primers as in PCR amplification and determined by the dideoxynucleotide chain termination method at the 1st BASE Sequencing, Shan Alan, Malasia (http://www.base-asia.com). The nucleotide sequence was analysed using the sequence match program of the Ribosomal Database Project II (RDP-II; http://rdp.cme.msu.edu) and GenBank DNA database search (www.ncbi.nlm.nih.gov/BLAST) (35). The closest relatives of the partial 16S rRNA gene sequences was evaluated. A similarity of ≥ 97% to 16S rRNA sequences of type strains was used as the criterion for identification.

5. THP-1 Cells and culture conditions

THP-1 cells are a human monocytic cell line (ATCC TIB-202, USA) originally isolated from a child with acute leukemia. They were purchased from the American Tissue Culture Collection (ATCC). These cells were suspension cells with monocytoid morphology (198).

5.1 Culture of THP-1 cells

These non-adherent cells were maintained in continuous culture with RPMI 1640 (with 2mM L-glutamine, 2000 mg/L D-glucose, 10mM HEPES, and 1.0 sodium pyruvate and 0.05 mM β-mercaptoethanol, adjusted to contain 2 g/L sodium bicarbonate) (Gibco-Invitrogen, USA), containing 10% heat-inactivated fetal bovine serum (FBS; Gibco-Invitrogen, USA). They were incubated at 37 ^{o}C in humidified 5% CO_2 incubator (BINDER, Germany). The doubling time for these cells under these conditions is approximately 48 h. These cells were monitored daily for morphology and growth characteristics.

5.2 THP-1 cell Sub-Culturing

THP-1 cells were maintained by the addition of RPMI 1640 fresh medium or replacement of medium. All cultures used sterile technique in a Vertical Laminar Flow workstation (Microflow, UK.). Cell cultures maintained between 5.0 x 10^4 to 8.0 x 10^5 viable cells/ml and the density did not exceed 1.0 x 10^6 cells/ml. Sub-culturing was performed every 2-3 days, depending on cell density. Sub-culture was done with inoculum of 2x10^4- 4x10^4 viable cell/ml. RPMI 1640 medium (Gibco-Invitrogen, USA) plus 10% heat-inactivated fetal bovine serum (Gibco-Invitrogen, USA) were used as medium to maintain cell culture which was incubated at 37^{o}C in a humidified 5% CO_2 environment. Since cells may change phenotypic and functional characteristics with prolonged passage, sub-cultures were made between 15-50 times. When THP-1 cells approached 50 passages, they were thawed from the new vial of frozen stock.

5.3 Freezing of THP-1 cells

The collection stocks of THP-1 cells were made in multiple frozen vials (10 or more vials) for subsequent use. The frozen stocks were prepared in cryoprotective medium which was consisted of RPMI 1640 plus 10% FBS supplemented with 5 % (V/V) dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA). The THP-1 stocks were kept at -80^{o}C overnight before the storage in liquid nitrogen vapor phase at -196^{o}C (Taylor-Wharton, USA). The THP-1 stock was thawed at 1-2 weeks after freezing to check cell viability and purity by using inverted microscope (Olympus, Japan).

5.4 Thawing of THP-1 cell

Collection vial of THP-1 from liquid nitrogen (-196^{o}C) tank was thawed as described in the manufacture's instruction. The vial was put in water bath (Gyromax TM 929, USA), by keeping the O-ring and cap out of the water, at 37^{o}C for approximately 2 min. After decontamination by spraying 70% ethanol, THP-1 cells were transferred into 9 ml RPMI 1640 plus 10% FBS (complete culture medium) in a conical centrifuge tube and centrifuged at 125xg for 5 min. After centrifugation, cell pellets were resuspended in 6 ml complete culture medium in tissure culture flask (25 cm^2 in size)(NUNC, Denmark) and incubated at 37 ^{o}C in 5% CO_2 incubator. The culture was checked for microbial contamination by using an inverted microscopic (Olympus, Japan). After incubation for several hours, the suspension was centrifuged at 125xg for 5-10 minutes and the pellet was resuspended in 6 ml of medium. Cell suspension was counted and checked for the viability, adjusted the density of cell to 5x10^{4}viable cell/ml and incubated horizontally at 37^{o}C in 5% CO_2 incubator (BINDER, Germany).

6. Assay for Immunomodulatory Effect of *Lactobacillus* isolates on TNF-α production in LPS-Activated THP-1 Monocytic Cells

6.1 Preparation of *Lactobacillus* Conditioned Media (LCM)

Lactobacillus isolates from gastric biopsies (17 isolates from group 1 patients with mild gastritis; 48 isolates from group 2 patients with severe gastritis and 24 isolates from group 3 patients with peptic ulcer) were tested. *L. saerimneri* strain TH58, TNF-α inhibitory strain was used as positive control and *L. reuteri* strain 9/7, non-TNF-α inhibitory strain, was used as negative control. These control strains were available in the laboratory. (Malai Taweechotipatr, Ph.D. Dissertation 2008). All *Lactobacillus* isolates recovered from frozen stocks (-80°C) were streaked on MRS agar and incubated anaerobically at 37°C for 24-48 h in anaerobic chamber (Concept Plus) or in anaerobic jar. A single colony of *Lactobacillus* was picked and re-streaked on MRS agar for 24 h. After incubation, a single colony of *Lactobacillus* was picked and inoculated in 5 ml of MRS broth and grown at 37 °C for 24 h in 15 ml- conical centrifuge tube (NUNC, USA).The OD_{600} of culture was determined by using spectrophotometer (Bio-Rad Smart Spec™ Plus) and adjusted to OD_{600} of 0.1. ($10x10^8$ cell/ml) in 10 ml of MRS broth and incubated for 24 and 48 h. After incubation, *Lactobacillus* culture was centrifuged in 15 ml round-bottom tubes at 4,000 rpm for 10 min. The supernatant was filtered through 0.22 μm pore size filter unit (Minisart, Germany). The supernatant of *Lactobacillus* without the cell pellet was called *Lactobacillus* condition media (LCM). The pH of LCM was adjusted by speed-vacuum drying (speed-vacuum, Savant instruments, USA) and resuspended in an equal volume of cell culture medium (RPMI 1640; Gibco-Invitrogen, USA). All LCM were stored at -20°C until analysis.

6.2 THP-1 Bioassay

THP-1 leukemic monocytic cells were maintained in RPMI 1640 Medium (Gibco-Invitrogen, USA) supplemented with 10 % heat-inactivated fetal bovine serum (FBS; Gibco-Invitrogen, USA). In-vitro bioassay was performed as previously described by Pena J A and Versalovic J (58). THP-1 cells were counted with hemocytometor (BOECO, Germany) under inverted microscope and diluted into fresh complete RPMI 1640 medium (RPMI 1640 plus 10% FBS) to a density of 2.5×10^5 cells/ml. THP-1 cell

suspension 200 µl were seeded into each well of a 96-well flat-bottom tissue culture plate (NUNCLON D, Denmark) and incubated at 37°C in humidified 5% CO_2 chamber for 10 minutes. Bioassay was started by incubating the cells with 10 µl (5% v/v) of MRS or complete RPMI 1640 or *Lactobacillus* conditioned media in the appropriate well and 5 µl of 100 ng/ml (final concentration) LPS of *E. coli* serotype O127:B8 (LPS; Sigma, USA) was added into the appropriate well. After 3.5 h incubation, supernatants were collected by into 1.5 ml centrifuge tubes and entrifugation at 1,000xg for 10 min in 4°C for TNF-α measurement. Cell viability was assessed by the trypan blue stain exclusion assay.

6.3 TNF-α measurement

TNF-α production from monocytic cells were detected with cytokine-specific sandwich quantitative enzyme-linked immunnosobent assay (sandwich ELISA) according to the manufacturer's instructions (TNF-α/TNF-SFII human DuoSet, R&D Systems, DY210, USA). Briefly, 96 well-microtiter plates (F96 CERT, MAXISORP, NUNC, Denmark) were coated with 100 µl per well of mouse anti-human TNF-α antibodies (R&D System, USA) as capturing antibodies diluted in phosphate buffer saline (PBS), pH 7.2-7.4. The ELISA plate was sealed and incubated overnight at room temperature or 4° C. After incubation, plates were aspirated and washed three times with 400 µl per well of wash buffer (PBS pH 7.2-7.4 containing with 0.05% Tween 20 (Amresco)) using a squirt bottle to remove excess capture antibody. After the last wash, any remaining wash buffer was removed by aspirating or inverting the plate and blotting it against clean paper towels. Plates were blocked with 300 µl per well of 1% (W/V) of A bovine serum albumin (BSA) (Sigma, USA) in PBS, pH 7.2-7.4 (reagent diluent (RD)) to reduce non-specific binding. Plates were incubated at room temperature for a minimum of 2 h and washed three times with 400 µl per well of wash buffer. The plates were added 100 µl of sample or standard in an appropriate well. The recombinant human TNF-α (R&D System, USA) was diluted seven point standards by use 2-fold serial dilutions at the concentration 1,000, 500, 250, 125, 62.5, 31.5, 15.625 and blank as reagent diluent. Plates were incubated overnight at room temperature. After incubation, the plates were aspirated and washed three times with wash buffer as described above. Biotinylated goat anti-human TNF-α (R&D System, USA) as detection antibodies diluted in reagent diluents (100 µl) was added in each well and incubated for 2 h at room temperature. After

incubation, the plates were aspirated and washed three times with wash buffer in above described. Streptavidin conjugated to horse radish-peroxidase dilued in reagent diluents (100 µl) were added to each well and incubated 20 min by avoiding the exposure to direct light. After incubaction, the plates were aspirated and washed three times with wash buffer as described above. Substrate solution (100 µl) as mixture of equal volume of reagent A (H_2O_2) and color reagent B (tetramethylbenzidine, TMB) was added to well plates and incubated for 20 min at room temperature by avoiding the exposure to direct light. Stop solution (100 µl of 2 N H_2SO_4; MERCK, Germany) was added in each well. Absorbance was determined with microplate reader at 450 nm. A standard curve was created based on the optical density and concentration of TNF using computer software. The result of TNF concentration was quantified from standard curve and shown as pg/ml of culture medium.

6.4 Statistical analyses

All experiments were performed in triplicate and the results were reported as mean and standard deviations (SD). The data were analysed using the Student's *t* test with one-tailed distribution and considered statistically significant at p-value ≤ 0.05. The statistical differences of the prevalence of TNF-suppressing *Lactobacillus* in each group of patients were analysed by using chi-square (x^2) test of SAS version 8. A p-value $\leq$ 0.05 was considered statistically significant. A binary logistic regression analysis of SPSS version 15 was perfomed using the prevalence of TNF-suppressing *Lactobacillus*, age and sex of the patients. A p-value ≤ 0.05 was considered statistically significant.

CHAPTER V

RESULTS

1. Cultivation and presumptive identification of *Lactobacillus* from gastric biopsies and throat swabs

Two hundred and seventy-two gastric biopsies and throat swabs were obtained from dyspeptic patients during the study period of 12 months from 25 October 2006 to 17 October 2007. These samples were categorized into 70 samples of group 1 patients with mild gastritis, 158 samples of group 2 patients with severe gastritis and 44 samples of group 3 patients with peptic ulcer. These samples were cultured in anaerobic condition. Bacterial colonies that were grown on MRS agar with different appearance (Figure 12) were picked and streaked for single colony isolation on new MRS agar plates. The most frequently found colonies were small to medium (2-2.5 mm), circular, with white transparent or turbid (Figure 13). Some colonies produce yellow pigment. A single pure colony was then subcultured for presumptive test by gram staining, catalase test and vancomycin susceptibility test.

A total of 106 and 193 isolates suspected of *Lactobacillus* were obtained from gastric biopsies and throat swabs, respectively. They were all gram-positive rods, catalase-negative and vancomycin susceptible or resistant (Figure 14). Different types of suspected *Lactobacillus* colonies isolated from gastric biopsy and throat of each patient were observed and summarized in Tables 7 and 8 and Figures 15 and 16, respectively. The majority of colonies were found to be one and two types.

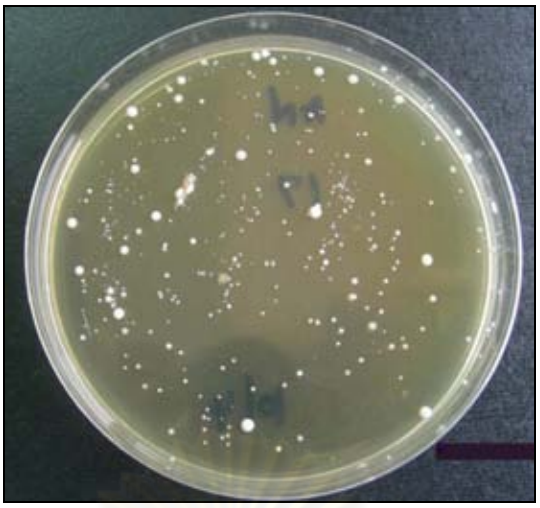


Figure 12. The primary plate of gastric biopsy as culture on MRS agar plates.

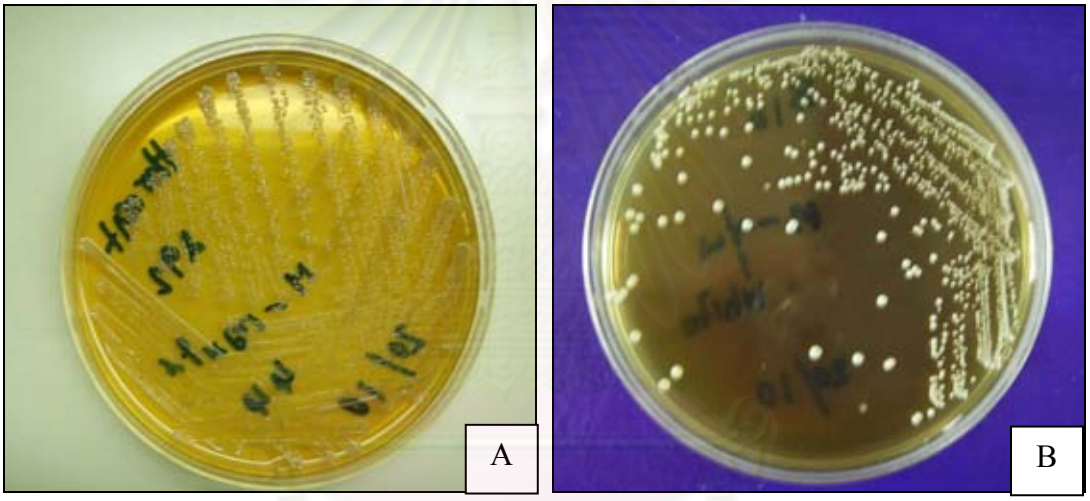


Figure 13. Colonies of *Lactobacillus* isolated from gastric biopsy samples of dyspeptic patients. (A) C olonies of *Lactobacillus* as w hite t ransparent, (B) C olonies of *Lactobacillus* as white turbid.

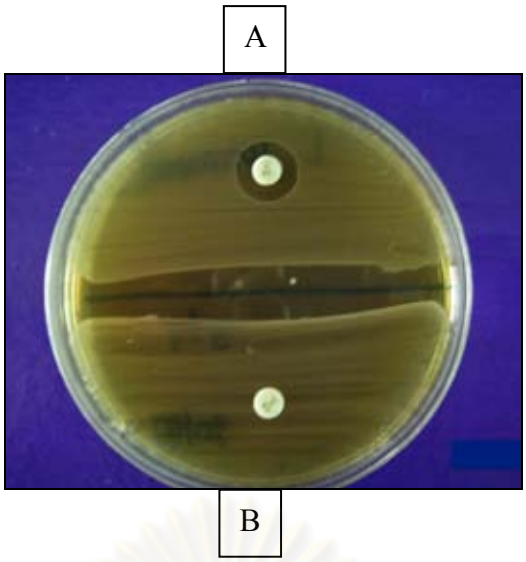


Figure 14. Vancomycin susceptibility of *Lactobacillus*. (A) *Lactobacillus* as susceptible (B) *Lactobacillus* as resistant.

Table 7. Number of different type of suspected *Lactobacillus* colonies isolated from gastric biopsy samples of each group of patients.

Group of dyspeptic patients	**Total samples**	**Number of samples with suspected *Lactobacillus***	**1 type**	**2 types**	**3 types**	**4 types**	**Total isolates**
Group 1	70	10	6	1	3	0	17
Group 2	158	32	20	10	0	2	48
Group 3	44	16	8	8	0	0	24

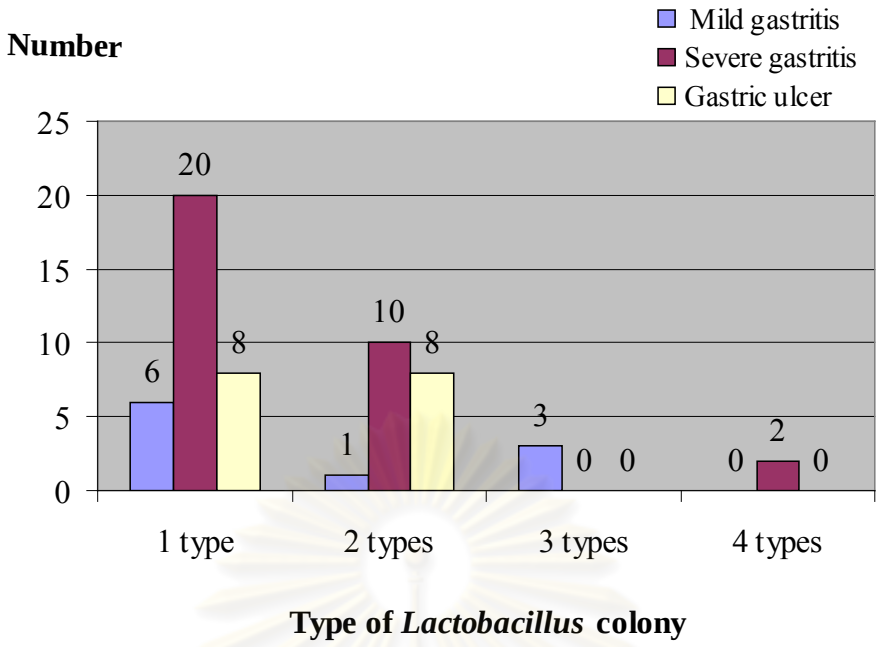


Figure 15. Number of different type of suspected *Lactobacillus* colonies isolated from gastric biopsy samples of each group of patients.

Table 8. Number of different type of suspected *Lactobacillus* colonies isolated from throat swabs samples of each group of patients.

Group of dyspeptic patients	**Total samples**	**Number of samples with suspected *Lactobacillus***	**1 type**	**2 type**	**3 type**	**4 type**	**5 type**	**Total isolates**
Group 1	75	27	14	6	6	0	1	49
Group 2	158	60	33	24	4	1	0	97
Group 3	44	20	9	11	1	0	0	34

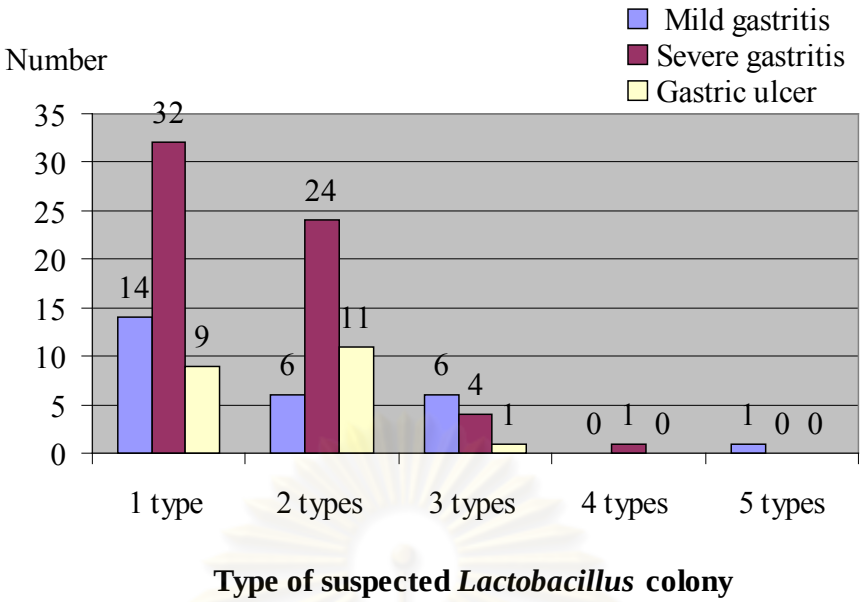


Figure 16. Number of different type of suspected *Lactobacillus* colonies isolated from throat swab samples of each group of patients.

2. Genotypic Identification of *Lactobacillus* isolates

To identify these suspected *Lactobacillus* isolates, 16S rRNA genes were amplified and sequenced. The genomic DNA of *Lactobacillus* isolates were extracted and amplified with two sets of primers: one set for the *Lactobacillus* group-specific amplification and the other set for universal amplification. Before amplification, two sets of primer were aligned with the 16S rRNA gene sequences of *Lactobacillus* spp. with Multalin program (http://bioinfo.genotoul.fr/multalin/multalin.html) in Figures 17 and 18. Amplification with *Lactobacillus* group-specific primers L341-F and L341R yieled a 341 bp PCR product as shown in Figure 19. Of the 106 suspected *Lactobacillus* isolates from gastric biopsies, 89 isolates (83.96%) were found to be positive for genus *Lactobacillus* amplification. Of the 193 suspected *Lactobacillus* isolates from throats swab, 180 isolates (93.26%) were found to be positive for genus *Lactobacillus* amplification.

Isolates with positive results (89 isolates from biopsies and 180 isolates from throat swabs) were subjected to amplification of complete 16S rRNA gene with a set of universal primers 16S-8F and 16S-1541R. The products of approximately 1,520 bp were

found as shown in Figure 20. The amplification products from gastric biopsies and throat swabs were sequenced and analysed with NCBI and RDP II database as shown in Tables 9-11 and 12-14, respectively. The similarity value closely related ≥ 97% to 16S rDNA sequences of type strains was used as the criterion for species identification.

The species of *Lactobacillus* isolates from gastric biopsies of patient groups 1, 2 and 3 were shown in Figures 21, 22 and 23, respectively. The summary of bacterial species found in gastric biopsies of dyspeptic patients was shown in Figure 24. Two of 89 isolates found in gastric biopsies were identified as *Weissella confusa*.

The species of *Lactobacillus* isolates from throat swabs of patient groups 1, 2 and 3 were shown in Figures 25, 26 and 27, respectively. The summary of bacterial species found in gastric biopsies of dyspeptic patients was shown in Figure 28. Three of 178 isolates found in throat swab were identified as *W. cibaria* and 3 isolates could not be identified, only matched with uncultered bacterium or Bacterium ii 1398.

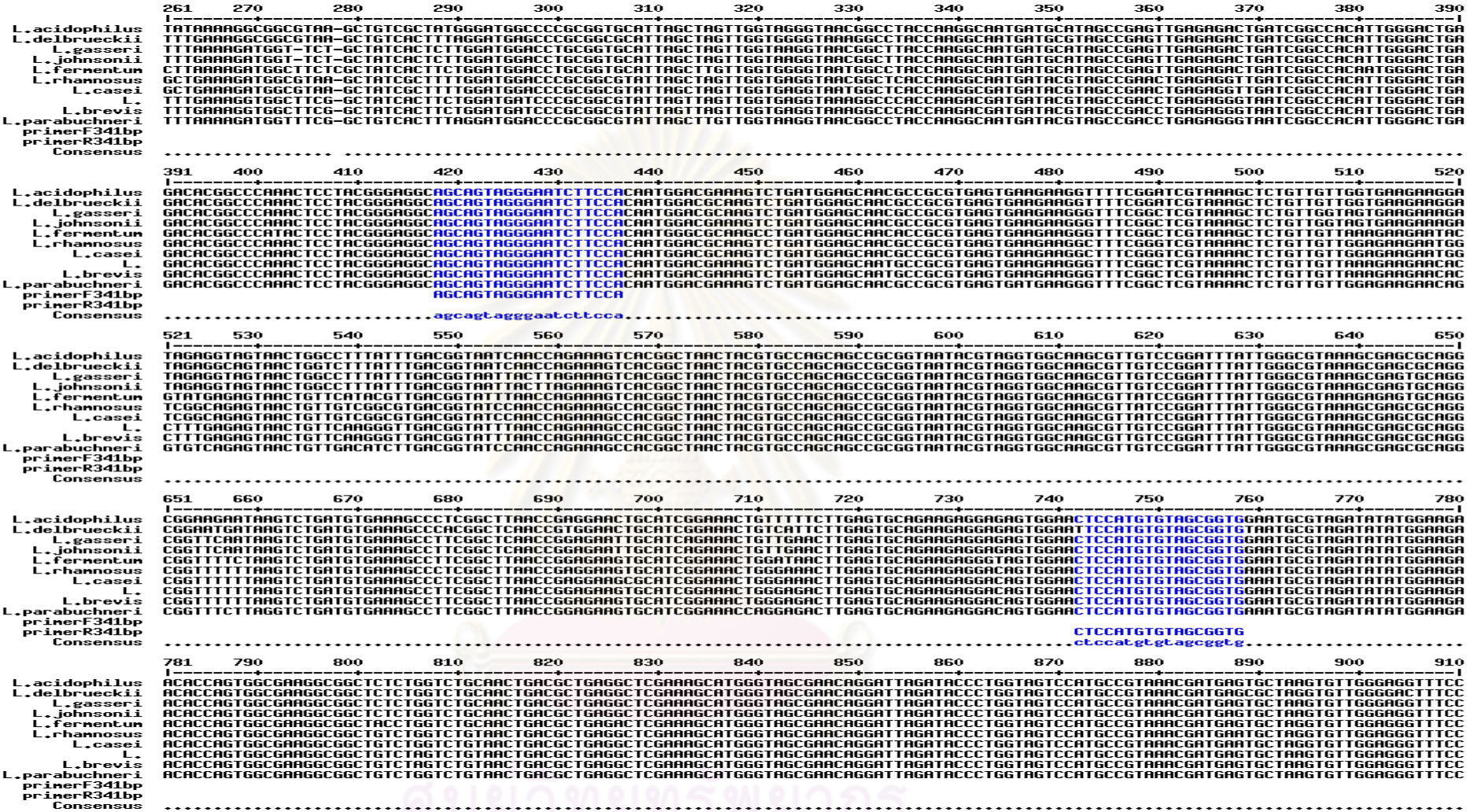
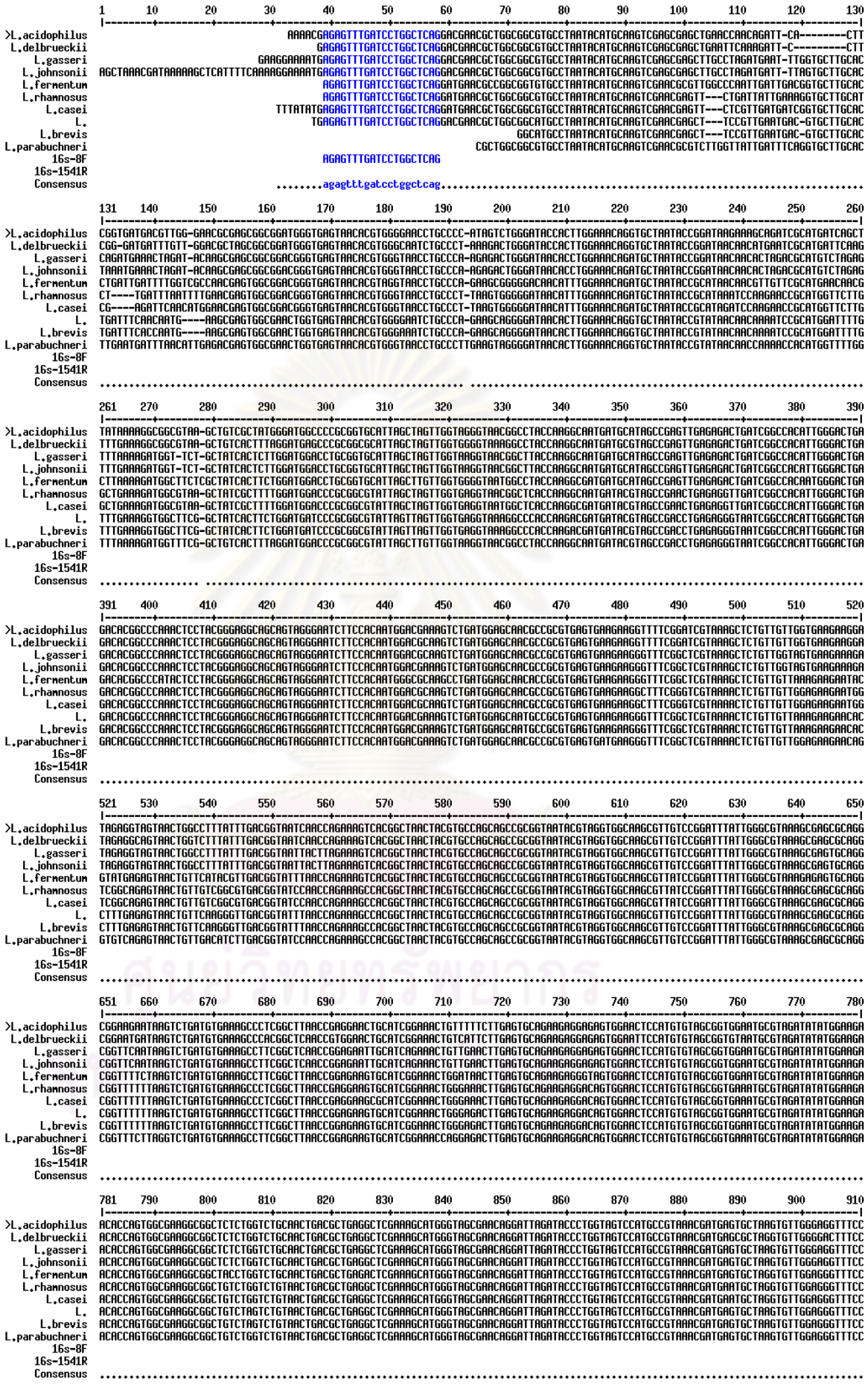


Figure 17. The alignment of *Lactobacillus* group-specific primers L341-F and L341-R with 16S rRNA gene sequence of *Lactobacillus* spp.



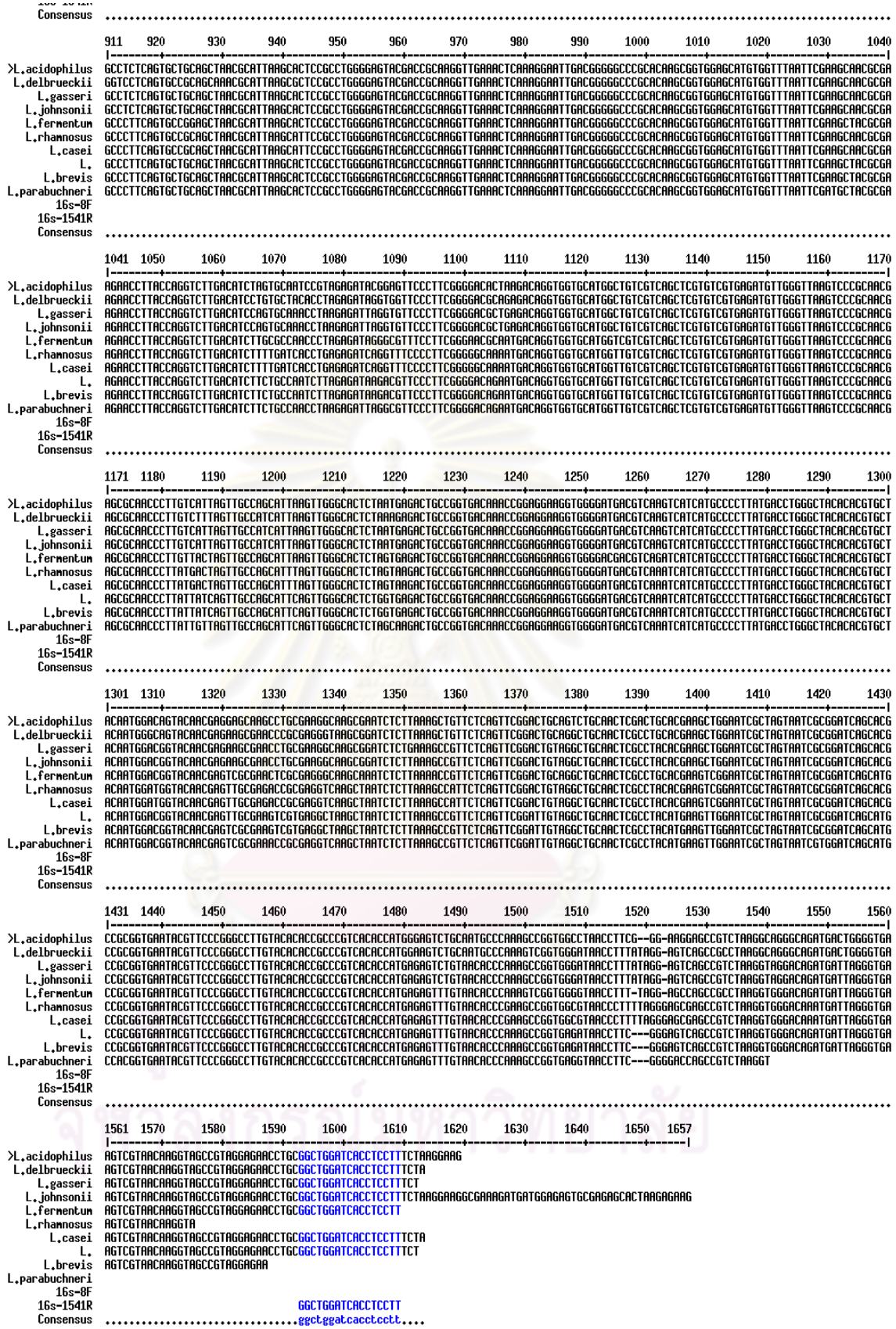


Figure 18. The alignment of universal primers 16S-8F and 16S-1541R with 16S rRNA gene sequence of *Lactobacillus* spp.

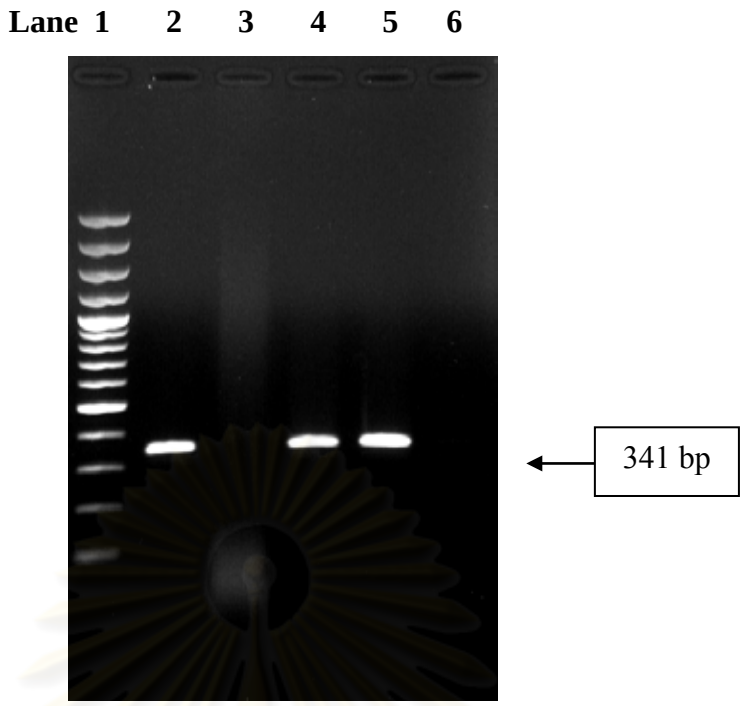


Figure 19. Amplification of 16S rRNA gene. Lane: 1, 100-bp DNA ledder plus; 2-4, DNA extract from suspected *Lactobacillus* isolates; 5, *Lactobacillus* DNA positive control; 6, negative control.

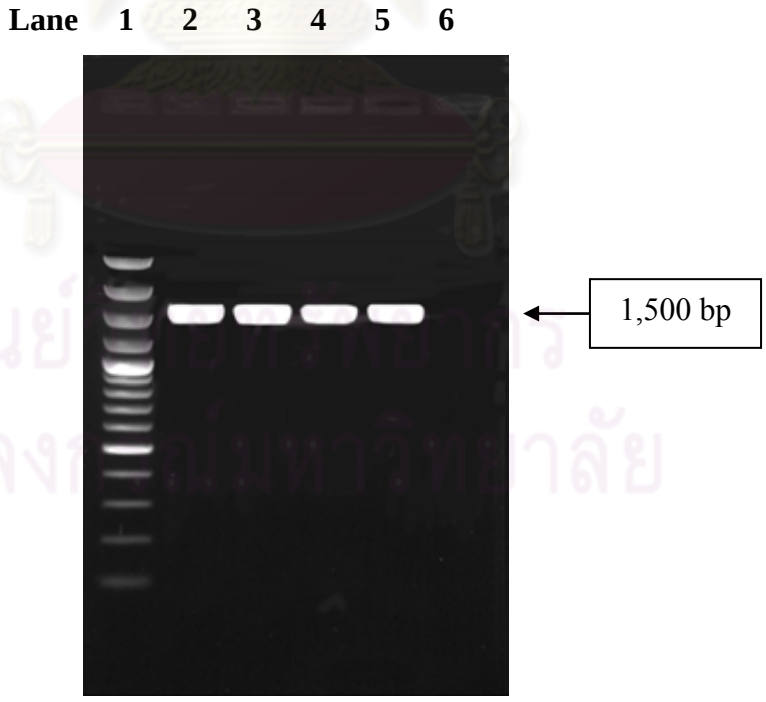


Figure 20. Amplification of complete 16S rRNA. Lane: 1, 100 bp DNA ledder plus; 2-4, DNA extract from suspected *Lactobacillus* isolates; 5, *Lactobacillus* DNA positive control; 6, negative control.

Table 9. Genotypic identification based on 16S r RNA gene sequencing of 16 *Lactobacillus* isolates from gastric biopsies of 9 dyspeptic patients with mild gastritis.

Subject and patient no.	***Lactobacillus* isolate**	**Match organism**	**% Similarity**
1	B13	*Lactobacillus casei* (NCBI)	99.0
(56)		*Lactobacillus paracasei* (NCBI)	99.0
		Lactobacillus casei (RDP)	99.6
2	B25	*Lactobacillus fermentum* (NCBI)	99.0
(77)		*Lactobacillus fermentum* (RDP)	100
3	B66	*Lactobacillus fermentum* (NCBI)	99.0
(163)		*Lactobacillus fermentum* (RDP)	100
	XB68	*Lactobacillus gasseri* (NCBI)	99.0
		Lactobacillus gasseri (RDP)	99.8
(184)	B71	*Weissella confusa* (NCBI)	99.0
		Weissella confusa (RDP)	98.8
4	B90	*Lactobacillus plantarum* (NCBI)	99.0
(217)		*Lactobacillus plantarum* (RDP)	100
5	B91	*Lactobacillus salivarius* (NCBI)	99.0
(225)		*Lactobacillus salivarius* (RDP)	99.8
	B92	*Lactobacillus fermentum* (NCBI)	99.0
		Lactobacillus fermentum (RDP)	99.2
	XB94	*Lactobacillus gasseri* (NCBI)	99.0
		Lactobacillus gasseri (RDP)	99.8
6	B93	*Lactobacillus oris* (NCBI)	99.0
(227)		*Lactobacillus oris* (RDP)	97.4
7	B101	*Lactobacillus salivarius* (NCBI)	100
(267)		*Lactobacillus salivarius* (RDP)	100
8	B105	*Lactobacillus fermentum*(NCBI)	98.0
(286)		*Lactobacillus fermentum* (RDP)	98.8

Table 9. Genotypic identification based on 16S r RNA gene sequencing of 16 *Lactobacillus* isolates from gastric biopsies of 9 dyspeptic patients with mild gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
	B106	*Lactobacillus casei* (NCBI)	99.0
		Lactobacillus paracasei (NCBI)	99.0
		Lactobacillus casei (RDP)	98.9
		Lactobacillus paracasei (RDP)	98.9
	B107	*Lactobacillus casei* (NCBI)	96.0
		Lactobacillus paracasei (NCBI)	96.0
		Lactobacillus casei (RDP)	97.7
		Lactobacillus paracasei (RDP)	97.7
		Lactobacillus rhamnosus (RDP)	97.7
9	B108	*Lactobacillus fermentum* (NCBI)	98.0
(292)		*Lactobacillus fermentum* (RDP)	98.5
	B109	*Lactobacillus salivarius* (NCBI)	98.0
		Lactobacillus salivarius (RDP)	99.3
	B110	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	98.6

Table 10. Genotypic identification based on 16S r RNA gene sequencing of 47 *Lactobacillus* isolates from gastric biopsies of 32 dyspeptic patients with severe gastritis.

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
1	B2	*Lactobacillus fermentum* (NCBI)	99.0
(26)		*Lactobacillus fermentum* (RDP)	99.0
2	B6	*Lactobacillus plantarum* (NCBI)	100
(30)		*Lactobacillus plantarum* (RDP)	100
3	B7	*Lactobacillus plantarum* (NCBI)	99.0
(43)		*Lactobacillus plantarum* (RDP)	100
4	B8	*Lactobacillus salivarius* (NCBI)	99.0
(44)		*Lactobacillus salivarius* (RDP)	99.5
5	B9	*Lactobacillus fermentum* (NCBI)	98.0
(47)		*Lactobacillus fermentum* (RDP)	98.6
6	B18	*Lactobacillus oris* (NCBI)	97.0
(68)		*Lactobacillus oris* (RDP)	96.8
	XB19	*Lactobacillus gasseri* (NCBI)	99.0
		Lactobacillus gasseri (RDP)	99.8
7	B20	*Lactobacillus fermentum* (NCBI)	99.0
(70)		*Lactobacillus fermentum* (RDP)	99.6
8	B21	*Lactobacillus salivarius* (NCBI)	99.0
(73)		*Lactobacillus salivarius* (RDP)	99.6
	B22	*Lactobacillus oris* (NCBI)	99.0
		Lactobacillus oris (RDP)	97.5
9	B29	*Lactobacillus fermentum* (NCBI)	99.0
(94)		*Lactobacillus fermentum* (RDP)	99.1
10	XB30	*Lactobacillus gasseri* (NCBI)	99.0
(96)		*Lactobacillus gasseri* (RDP)	99.8
	B35	*Lactobacillus fermentum* (NCBI)	99.0
		Lactobacillus fermentum (RDP)	100

Table 10. Genotypic identification based on 16S r RNA gene sequencing of 47 *Lactobacillus* isolates from gastric biopsies of 32 dyspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
11	B31	*Lactobacillus fermentum* (NCBI)	99.0
(95)		*Lactobacillus fermentum* (RDP)	98.4
12	B38	*Lactobacillus fermentum* (NCBI)	99.0
(110)		*Lactobacillus fermentum* (RDP)	100
	B39	*Lactobacillus fermentum* (NCBI)	99.0
		Lactobacillus fermentum (RDP)	100
13	XB41	*Lactobacillus gasseri* (NCBI)	99.0
(105)		*Lactobacillus gasseri* (RDP)	99.8
14	B42	*Lactobacillus fermentum* (NCBI)	99.0
(120)		*Lactobacillus fermentum* (RDP)	97.4
15	XB45	*Lactobacillus gasseri* (NCBI)	99.0
(121)		*Lactobacillus gasseri* (RDP)	99.8
16	B46	*Lactobacillus fermentum* (NCBI)	99.0
(132)		*Lactobacillus fermentum* (RDP)	99.9
	B47	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	99.5
17	XB48	*Lactobacillus gasseri* (NCBI)	99.0
(135)		*Lactobacillus gasseri* (RDP)	99.8
18	XB49	*Lactobacillus gasseri* (NCBI)	99.0
(137)		*Lactobacillus gasseri* (RDP)	99.8
19	B53	*Lactobacillus salivarius* (NCBI)	99.0
(153)		*Lactobacillus salivarius* (RDP)	99.5
	B54	*Lactobacillus mucosae* (NCBI)	99.0
		Lactobacillus mucosae (RDP)	100
20	B55	*Lactobacillus salivarius* (NCBI)	99.0
(155)		*Lactobacillus salivarius* (RDP)	99.5

Table 10. Genotypic identification based on 16S r RNA gene sequencing of 47 *Lactobacillus* isolates from gastric biopsies of 32 dyspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
21	XB58	*Lactobacillus gasseri* (NCBI)	99.0
(154)		*Lactobacillus gasseri* (RDP)	99.2
	B64	*Lactobacillus plantarum* (NCBI)	99.0
		Lactobacillus pentosus (NCBI)	99.0
		Lactobacillus plantarum (RDP)	100
	B59	*Weissella confusa* (NCBI)	99.0
		Weissella confusa (RDP)	98.6
	B60	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	99.0
22	B67	*Lactobacillus plantarum* (NCBI)	99.0
(165)		*Lactobacillus plantarum* (RDP)	99.4
23	B70	*Lactobacillus plantarum* (NCBI)	99.0
		Lactobacillus plantarum (RDP)	100
24	B72	*Lactobacillus fermentum* (NCBI)	99.0
(185)		*Lactobacillus fermentum* (RDP)	99.0
	B73	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	98.9
25	B74	*Lactobacillus salivarius* (NCBI)	99.0
(190)		*Lactobacillus salivarius* (RDP)	99.5
	B75	*Lactobacillus fermentum* (NCBI)	99.0
		Lactobacillus fermentum (RDP)	99.6
	XB77	*Lactobacillus gasseri* (NCBI)	99.0
		Lactobacillus gasseri (RDP)	99.8
	B78	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	99.5

Table 10. Genotypic identification based on 16S r RNA gene sequencing of 47 *Lactobacillus* isolates from gastric biopsies of 32 dyspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
26	B76	*Lactobacillus fermentum* (NCBI)	99.0
(187)		*Lactobacillus fermentum* (RDP)	99.6
27	B79	*Lactobacillus mucosae* (NCBI)	99.0
(192)		*Lactobacillus mucosae* (RDP)	97.1
	BT121	*Lactobacillus mucosae* (NCBI)	97.0
		Lactobacillus mucosae (RDP)	95.7
28	B82	*Lactobacillus fermentum* (NCBI)	99.0
(200)		*Lactobacillus fermentum* (RDP)	99.4
	B83	*Lactobacillus fermentum* (NCBI)	98.0
		Lactobacillus fermentum (RDP)	96.7
29	B87	*Lactobacillus plantarum* (NCBI)	99.0
(210)		*Lactobacillus pentosus* (NCBI)	99.0
		Lactobacillus plantarum (RDP)	100
30	XB95	*Lactobacillus gasseri* (NCBI)	99.0
(235)		*Lactobacillus gasseri* (RDP)	99.8
31	XB96	*Lactobacillus gasseri* (NCBI)	99.0
(232)		*Lactobacillus gasseri* (RDP)	99.8
32	B102	*Lactobacillus salivarius* (NCBI)	99.0
(276)		*Lactobacillus salivarius* (RDP)	98.6
	B103	*Lactobacillus rhamnosus* (NCBI)	98.0
		Lactobacillus casei (NCBI)	98.0
		Lactobacillus paracasei (NCBI)	98.0
		Lactobacillus rhamnosus (RDP)	99.5

Table 11. Genotypic identification based on 16S r RNA gene sequencing of 24 *Lactobacillus* isolates from gastric biopsies of 16 dyspeptic patients with gastric ulcer and duodenum ulcer (DU);** Dyspeptic patients as DU.

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
1	XB7	*Lactobacillus plantarum* (NCBI)	99.0
(27)		*Lactobacillus pentosus* (NCBI)	99.0
		Lactobacillus plantarum (RDP)	99.3
2	B4/2	*Lactobacillus salivarius* (NCBI)	99.0
(28)		*Lactobacillus salivarius* (RDP)	99.5
	B5	*Lactobacillus fermentum* (NCBI)	99.0
		Lactobacillus fermentum (RDP)	99.4
3	B14	*Lactobacillus fermentum* (NCBI)	96.0
(57)		*Lactobacillus fermentum* (RDP)	96.3
4	B15	*Lactobacillus mucosae* (NCBI)	99.0
**(67)		*Lactobacillus mucosae* (RDP)	99.5
	B16	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	99.5
5	B23	*Lactobacillus salivarius* (NCBI)	99.0
(76)		*Lactobacillus salivarius* (RDP)	99.5
	B24	*Lactobacillus fermentum* (NCBI)	99.0
		Lactobacillus fermentum (RDP)	99.2
6	B26	*Lactobacillus mucosae* (NCBI)	99.0
(85)		*Lactobacillus mucosae* (RDP)	100
7	B32	*Lactobacillus salivarius* (NCBI)	99.0
(99)		*Lactobacillus salivarius* (RDP)	100
	B33	*Lactobacillus fermentum* (NCBI)	99.0
		Lactobacillus fermentum (RDP)	100
8	B36	*Lactobacillus agilis* (NCBI)	98.0
(108)		*Lactobacillus* sp. 52A (RDP)	99.4
		Lactobacillus agilis (RDP)	95.7

Table 11. Genotypic identification based on 16S r RNA gene sequencing of 24 *Lactobacillus* isolates from gastric biopsies of 16 dyspeptic patients with gastric ulcer and duodenum ulcer (DU);** Dyspeptic patients as DU. (Continued)

Subject and patient no.	***Lactobacillus* isolate**	**Match organism**	**% Similarity**
	B37	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	99.5
9	XB40	*Lactobacillus gasseri* (NCBI)	99.0
(109)		*Lactobacillus gasseri* (RDP)	98.8
10	B43	*Lactobacillus salivarius* (NCBI)	99.0
**(123)		*Lactobacillus salivarius* (RDP)	98.8
	B44	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	95.0
11	B52	*Lactobacillus salivarius* (NCBI)	99.0
(146)		*Lactobacillus salivarius* (RDP)	98.8
12	B57	*Lactobacillus murinus* (NCBI)	99.0
(156)		*Lactobacillus murinus* (RDP)	96.8
13	B61	*Lactobacillus fermentum* (NCBI)	99.0
(158)		*Lactobacillus fermentum* (RDP)	99.4
	B62	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	99.5
14	B85	*Lactobacillus salivarius* (NCBI)	99.0
(206)		*Lactobacillus salivarius* (RDP)	99.5
	B84	*Lactobacillus fermentum* (NCBI)	99.0
		Lactobacillus fermentum (RDP)	99.6
15	B98	*Lactobacillus fermentum* (NCBI)	99.0
(250)		*Lactobacillus fermentum* (RDP) (F)	97.4
16	B99	*Lactobacillus fermentum* (NCBI)	99.0
(257)		*Lactobacillus fermentum* (RDP)	99.6

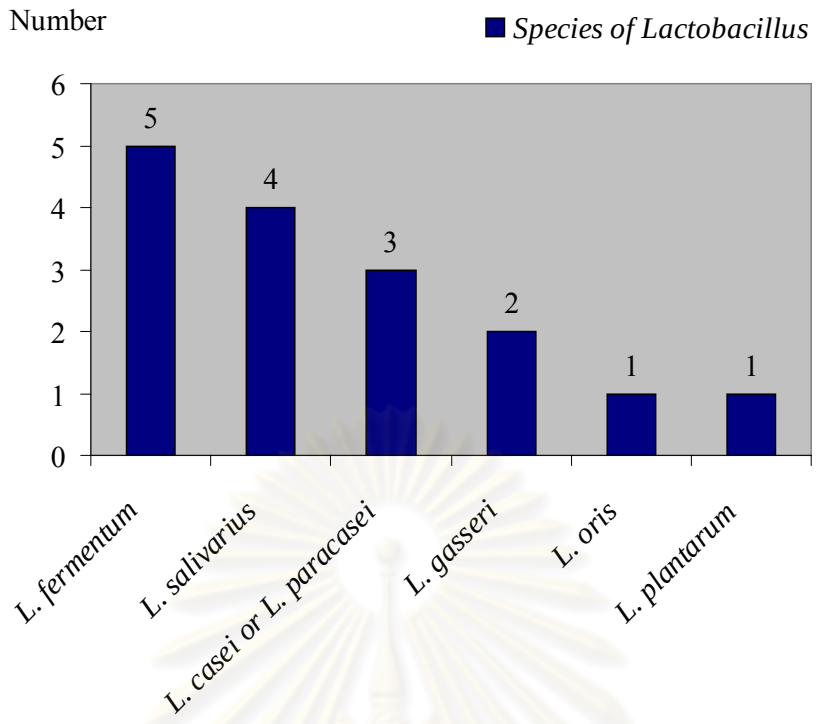


Figure 21. The species of *Lactobacillus* isolates from gastric biopsies of mild gastritis patients as identified by PCR and DNA sequencing.

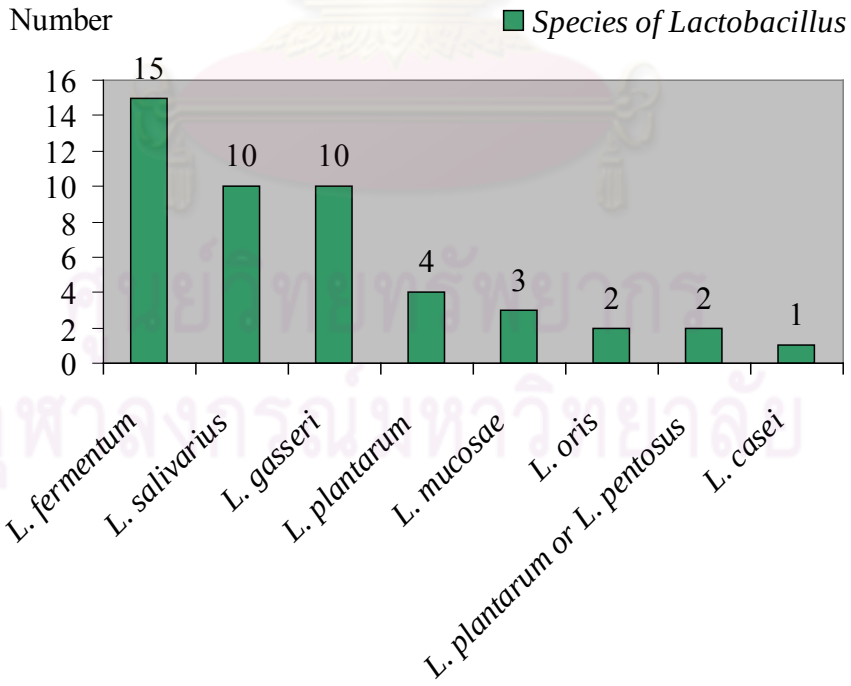


Figure 22. The species of *Lactobacillus* isolates from gastric biopsies of severe gastritis patients as identified by PCR and DNA sequencing.

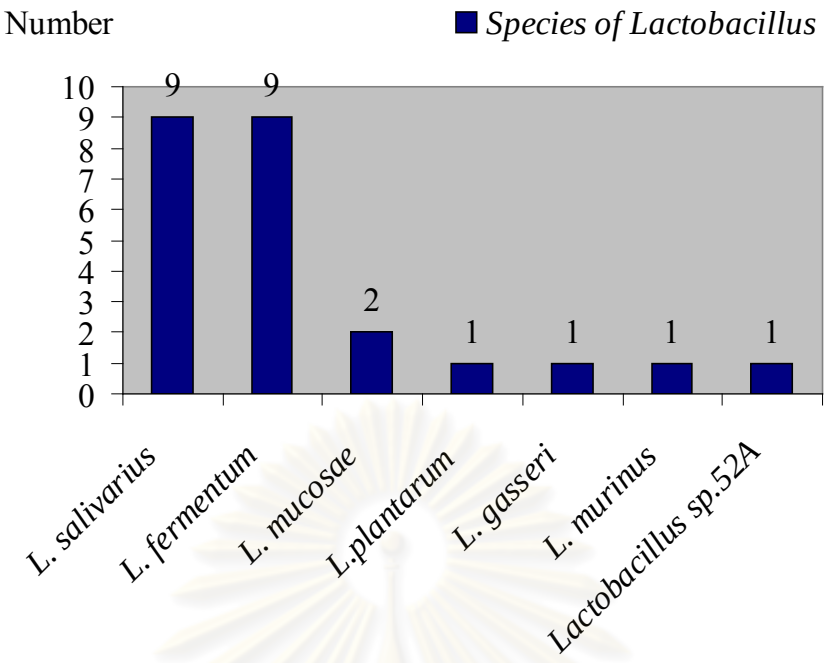


Figure 23. The species of *Lactobacillus* isolates from gastric biopsies of peptic ulcer patients as identified by PCR and DNA sequencing.

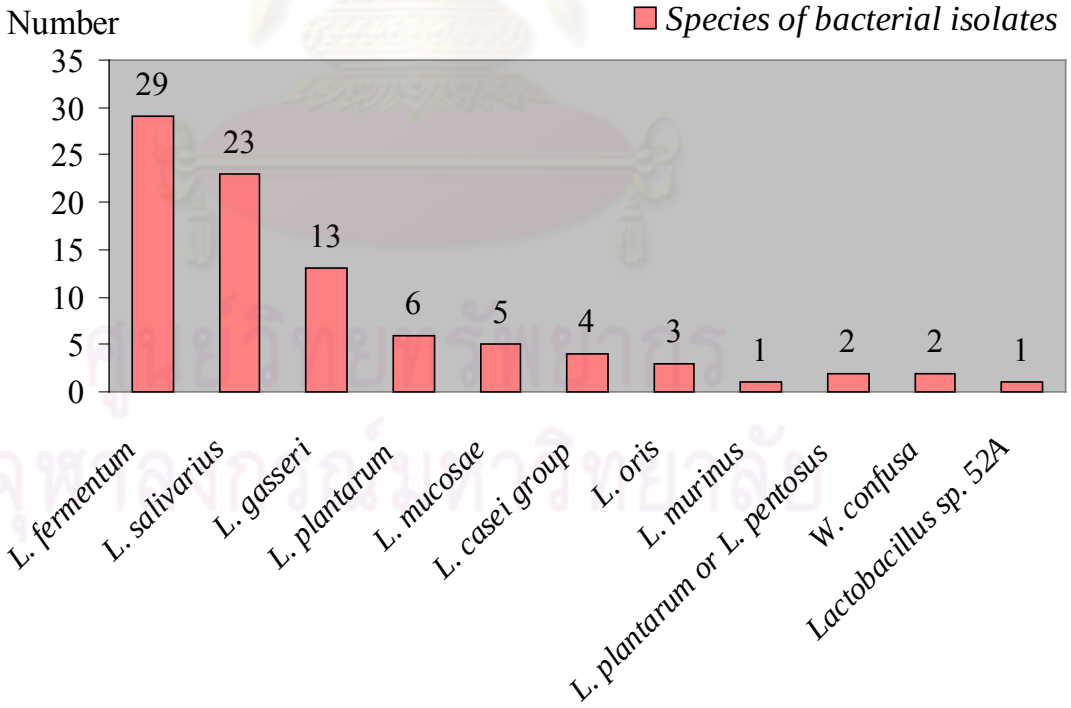


Figure 24. The species of bacterial isolates from gastric biopsies of dyspeptic patients.

Table 12. Genotypic identification based on 16S r RNA gene sequencing of 48 *Lactobacillus* isolates from throat swabs of 25 dyspeptic patients with mild gastritis.

Subject and patient no.	***Lactobacillus* isolate**	**Match organism**	**% Similarity**
1	T2/2	*Lactobacillus fermentum* (NCBI)	99.0
(14)		*Lactobacillus fermentum* (RDP)	97.1
2	T17	*Lactobacillus salivarius* (NCBI)	99.0
(39)		*Lactobacillus salivarius* (RDP)	99.2
3	T19	*Lactobacillus salivarius* (NCBI)	99.0
(45)		*Lactobacillus salivarius* (RDP)	99.0
4	T21	*Lactobacillus casei* (NCBI)	97.0
(56)		*Lactobacillus paracasei* (NCBI)	97.0
		Lactobacillus casei (RDP)	99.1
		Lactobacillus paracasei (RDP)	99.1
5	T37	*Lactobacillus fermentum* (NCBI)	97.0
(88)		*Lactobacillus fermentum* (RDP)	98.3
6	T49	*Lactobacillus salivarius* (NCBI)	99.0
(100)		*Lactobacillus salivarius* (RDP)	99.1
7	T62	*Lactobacillus salivarius* (NCBI)	100
(112)		*Lactobacillus salivarius* (RDP)	98.4
(115)	T65	*Weissella cibaria* (NCBI)	95.0
		Weissella cibaria (RDP)	96.0
8	T69	*Lactobacillus salivarius* (NCBI)	99.0
(122)		*Lactobacillus salivarius* (RDP)	99.0
9	T93	*Lactobacillus fermentum* (NCBI)	96.0
(162)		*Lactobacillus fermentum* (RDP)	97.5
	T100	*Lactobacillus plantarum* (NCBI)	98.0
		Lactobacillus plantarum (RDP)	99.7
10	T97	*Lactobacillus fermentum* (NCBI)	98.0
(163)		*Lactobacillus fermentum* (RDP)	99.8

Table 12. Genotypic identification based on 16S rRNA gene sequencing of 48 *Lactobacillus* isolates from throat swabs of 25 dyspeptic patients with mild gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
	T95	*Lactobacillus mucosae* (NCBI)	97.0
		Lactobacillus mucosae (RDP)	98.4
	T96	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	97.7
11	T99	*Lactobacillus plantarum* (NCBI)	97.0
(164)		*Lactobacillus plantarum* (RDP)	98.0
12	T101	*Lactobacillus mucosae* (NCBI)	99.0
		Lactobacillus mucosae (RDP)	94.2
(189)	T117/1	Uncultured bacterium (NCBI)	98.0
		Bacterium ii1389 (RDP)	97.5
13	T119	*Lactobacillus fermentum* (NCBI)	95.0
(191)		*Lactobacillus fermentum* (RDP)	97.5
	T120	*Lactobacillus salivarius* (NCBI)	95.0
		Lactobacillus salivarius (RDP)	98.9
	T122	*Lactobacillus salivarius* (NCBI)	97.0
		Lactobacillus salivarius (RDP)	94.7
	T123	*Lactobacillus fermentum* (NCBI)	98.0
		Lactobacillus fermentum (RDP)	99.4
	T124	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	98.9
14	T133	*Lactobacillus fermentum* (NCBI)	94.0
(205)		*Lactobacillus* sp. (RDP)	96.5
		Lactobacillus fermentum (RDP)	96.0
15	T141	*Lactobacillus casei* (NCBI)	97.0
(214)		*Lactobacillus paracasei* (NCBI)	97.0
		Lactobacillus zeae (NCBI)	97.0

Table 12. Genotypic identification based on 16S rRNA gene sequencing of 48 *Lactobacillus* isolates from throat swabs of 25 dyspeptic patients with mild gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
		Lactobacillus rhamnosus (NCBI)	97.0
		Lactobacillus casei (RDP)	99.3
		Lactobacillus paracasei (RDP)	99.3
		Lactobacillus rhamnosus (RDP)	99.3
	T142	*Lactobacillus casei* (NCBI)	97.0
		Lactobacillus paracasei (NCBI)	97.0
		Lactobacillus zeae (NCBI)	97.0
		Lactobacillus rhamnosus (NCBI)	97.0
		Lactobacillus casei (RDP)	100
		Lactobacillus paracasei (RDP)	100
16	XT143	*Lactobacillus gasseri* (NCBI)	96.0
(212)		*Lactobacillus gasseri* (RDP)	100
	T144	*Lactobacillus oris* (NCBI)	98.0
		Lactobacillus oris (RDP)	99.3
	T145	*Lactobacillus salivarius* (NCBI)	95.0
		Lactobacillus salivarius (RDP)	98.4
17	T149	*Lactobacillus fermentum* (NCBI)	97.0
(224)		*Lactobacillus fermentum* (RDP)	98.0
	T150	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	99.5
18	T152	*Lactobacillus salivarius* (NCBI)	96.0
(225)		*Lactobacillus salivarius* (RDP)	97.5
	T153	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	98.4
19	T154	*Lactobacillus salivarius* (NCBI)	98.0
(227)		*Lactobacillus salivarius* (RDP)	98.0

Table 12. Genotypic identification based on 16S rRNA gene sequencing of 48 *Lactobacillus* isolates from throat swabs of 25 dyspeptic patients with mild gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
	T155	*Lactobacillus vaginalis* (NCBI)	98.0
		Lactobacillus vaginalis (RDP)	97.1
20 (243)	T163	*Lactobacillus fermentum* (NCBI)	98.0
		Lactobacillus fermentum (RDP)	90.2
	T165	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	97.5
	T166	*Lactobacillus plantarum* (NCBI)	98.0
		Lactobacillus plantarum (RDP)	98.7
	T167	*Lactobacillus salivarius* (NCBI)	96.0
		Lactobacillus salivarius (RDP)	98.6
21 (267)	T181	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	96.8
	T182	*Lactobacillus salivarius* (NCBI)	98.0
		Lactobacillus salivarius (RDP)	99.5
	T183	*Lactobacillus delbrueckii* (NCBI)	96.0
		Lactobacillus delbrueckii (RDP)	91.2
22 (286)	T186	*Lactobacillus fermentum* (NCBI)	96.0
		Lactobacillus fermentum (RDP)	98.9
	T187	*Lactobacillus casei* (NCBI)	97.0
		Lactobacillus paracasei (NCBI)	97.0
		Lactobacillus zeae (NCBI)	97.0
		Lactobacillus rhamnosus (NCBI)	97.0
		Lactobacillus casei (RDP)	98.9
		Lactobacillus paracasei (RDP)	98.9
		Lactobacillus rhamnosus (RDP)	98.9

Table 12. Genotypic identification based on 16S r RNA gene sequencing of 48 *Lactobacillus* isolates from throat swabs of 25 dyspeptic patients with mild gastritis. (Continued)

Subject and patient no.	***Lactobacillus* isolate**	**Match organism**	**% Similarity**
	T188	*Lactobacillus casei* (NCBI)	98.0
		Lactobacillus paracasei (NCBI)	98.0
		Lactobacillus zeae (NCBI)	98.0
		Lactobacillus rhamnosus (NCBI)	98.0
		Lactobacillus casei (RDP)	99.9
		Lactobacillus paracasei (RDP)	99.9
		Lactobacillus rhamnosus (RDP)	99.9
23	T189	*Lactobacillus salivarius* (NCBI)	98.0
(290)		*Lactobacillus salivarius* (RDP)	99.5
	T190	*Lactobacillus fermentum* (NCBI)	98.0
		Lactobacillus fermentum (RDP)	99.3
24	T191	*Lactobacillus salivarius* (NCBI)	98.0
(291)		*Lactobacillus salivarius* (RDP)	98.8
25	T192	*Lactobacillus fermentum* (NCBI)	98.0
(292)		*Lactobacillus fermentum* (RDP)	99.1
	T193	*Lactobacillus salivarius* (NCBI)	98.0
		Lactobacillus salivarius (RDP)	98.9
	T194	*Lactobacillus salivarius* (NCBI)	98.0
		Lactobacillus salivarius (RDP)	99.3

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis.

Subject and patient no.	***Lactobacillus* isolate**	**Match organism**	**% Similarity**
1	T1	*Lactobacillus salivarius* (NCBI)	97.0
(12)		*Lactobacillus salivarius* (RDP)	97.0
2	XT4	*Lactobacillus fermentum* (NCBI)	95.0
(21)		*Lactobacillus fermentum* (RDP)	92.4
3	T5	*Lactobacillus fermentum* (NCBI)	96.0
(26)		*Lactobacillus fermentum* (RDP)	99.1
	T6	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	100
4	T12	*Lactobacillus fermentum* (NCBI)	98.0
(33)		*Lactobacillus fermentum* (RDP)	100
	T13	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	99.6
5	T14	*Lactobacillus salivarius* (NCBI)	99.0
(37)		*Lactobacillus salivarius* (RDP)	100
	T15	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	99.0
	T16	*Lactobacillus salivarius* (NCBI)	100
		Lactobacillus salivarius (RDP)	100
6	T18	*Lactobacillus salivarius* (NCBI)	97.0
(44)		*Lactobacillus salivarius* (RDP)	99.2
7	T20	*Lactobacillus casei* (NCBI)	95.0
(46)		*Lactobacillus paracasei* (NCBI)	95.0
		Lactobacillus casei (RDP)	98.6
		Lactobacillus paracasei (RDP)	98.6
		Lactobacillus rhamnosus (RDP)	98.6

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
8	T23	*Lactobacillus* sp. (NCBI)	98.0
(58)		*Lactobacillus agilis* (NCBI)	97.0
		Bacterium ii1389 (RDP)	95.4
9	T26	*Lactobacillus salivarius* (NCBI)	98.0
(70)		*Lactobacillus salivarius* (RDP)	100
10	T27	*Lactobacillus oris* (NCBI)	95.0
(68)		*Lactobacillus oris* (RDP)	99.9
11	T28	*Lactobacillus salivarius* (NCBI)	97.0
(72)		*Lactobacillus salivarius* (RDP)	98.9
12	T32/1	*Lactobacillus casei* (NCBI)	95.0
(82)		*Lactobacillus paracasei* (NCBI)	95.0
		Lactobacillus casei (RDP)	99.6
		Lactobacillus paracasei (RDP)	99.6
		Lactobacillus rhamnosus (RDP)	99.6
	T32/2	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	99.0
13	XT35	*Lactobacillus gasseri* (NCBI)	99.0
		Lactobacillus gasseri (RDP)	97.9
14	T36	*Lactobacillus reuteri* (NCBI)	99.0
(87)		*Lactobacillus reuteri* (RDP)	98.1
15	T38	*Lactobacillus salivarius* (NCBI)	98.0
(92)		*Lactobacillus salivarius* (RDP)	100
16	T40	*Lactobacillus salivarius* (NCBI)	97.0
(94)		*Lactobacillus salivarius* (RDP)	97.2
	T41	*Lactobacillus fermentum* (NCBI)	95.0
		Lactobacillus fermentum (RDP)	98.5

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
17	T42	*Lactobacillus salivarius* (NCBI)	96.0
(95)		*Lactobacillus salivarius* (RDP)	97.3
18	T43	*Lactobacillus salivarius* (NCBI)	99.0
(96)		*Lactobacillus salivarius* (RDP)	98.9
	XT44/1	*Lactobacillus fermentum* (NCBI)	96.0
		Lactobacillus fermentum (RDP)	98.2
	T44/2	*Lactobacillus delbrueckii* (NCBI)	95.0
		Lactobacillus delbrueckii (RDP)	96.5
19	T46	*Lactobacillus fermentum* (NCBI)	96.0
(97)		*Lactobacillus fermentum* (RDP)	97.3
20	T50	*Lactobacillus mucosae* (NCBI)	97.0
(101)		*Lactobacillus mucosae* (RDP)	99.8
21	T54	*Lactobacillus fermentum* (NCBI)	95.0
(98)		*Lactobacillus fermentum* (RDP)	99.5
	T55	*Lactobacillus fermentum* (NCBI)	94.0
		Lactobacillus fermentum (RDP)	96.7
22	T53	*Lactobacillus fermentum* (NCBI)	97.0
(103)		*Lactobacillus fermentum* (RDP)	99.7
23	T56	*Lactobacillus mucosae* (NCBI)	96.0
(104)		*Lactobacillus mucosae* (RDP)	97.9
24	T57	*Lactobacillus fermentum* (NCBI)	95.0
(105)		*Lactobacillus fermentum* (RDP)	95.6
	T64	*Lactobacillus fermentum* (NCBI)	95.0
		Lactobacillus fermentum (RDP)	97.2

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
25	T61	*Lactobacillus mucosae* (NCBI)	98.0
(113)		*Lactobacillus mucosae* (RDP)	98.9
26	T66	*Lactobacillus fermentum* (NCBI)	97.0
(120)		*Lactobacillus fermentum* (RDP)	98.8
27	T67	*Lactobacillus salivarius* (NCBI)	96.0
(121)		*Lactobacillus salivarius* (RDP)	98.2
	T68	*Lactobacillus salivarius* (NCBI)	97.0
		Lactobacillus salivarius (RDP)	99.3
28	T73	*Lactobacillus mucosae* (NCBI)	97.0
(128)		*Lactobacillus mucosae* (RDP)	98.8
29	T75	*Lactobacillus salivarius* (NCBI)	98.0
(133)		*Lactobacillus salivarius* (RDP)	98.8
30	T76	*Lactobacillus salivarius* (NCBI)	96.0
(137)		*Lactobacillus salivarius* (RDP)	99.7
31	T79	*Lactobacillus rhamnosus* (NCBI)	96.0
(149)		*Lactobacillus casei* (NCBI)	96.0
		Lactobacillus paracasei (NCBI)	96.0
		Lactobacillus zeae (NCBI)	96.0
		Lactobacillus casei (RDP)	99.1
		Lactobacillus paracasei (RDP)	99.1
		Lactobacillus rhamnosus (RDP)	99.1
	T80	*Lactobacillus fermentum* (NCBI)	96.0
		Lactobacillus fermentum (RDP)	99.5

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
32	T81	*Lactobacillus salivarius* (NCBI)	99.0
(151)		*Lactobacillus salivarius* (RDP)	99.0
	T82	*Lavtobacillus fermentum* (NCBI	95.0
		Lactobacillus fermentum (RDP)	98.4
33	T83	*Lactobacillus salivarius* (NCBI)	96.0
(153)		*Lactobacillus salivarius* (RDP)	97.3
	T84	*Lactobacillus mucosae* (NCBI)	97.0
		Lactobacillus mucosae (RDP)	99.0
34	T85	*Lactobacillus salivarius* (NCBI)	100
(155)		*Lactobacillus salivarius* (RDP)	99.8
35	T86	*Lactobacillus salivarius* (NCBI)	99.0
(154)		*Lactobacillus salivarius* (RDP)	99.4
	T87	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	99.2
36	T89	*Lactobacillus salivarius* (NCBI)	96.0
(157)		*Lactobacillus salivarius* (RDP)	97.5
	XT92	*Lactobacillus gasseri* (NCBI)	97.0
		Lactobacillus gasseri (RDP)	99.4
37	T98	*Lactobacillus fermentum* (NCBI)	94.0
(166)		*Lactobacillus fermentum* (RDP)	98.1
38	T104	*Lactobacillus plantarum* (NCBI)	96.0
(172)		*Lactobacillus plantarum* (RDP)	94.6
	T105	*Lactobacillus fermentum* (NCBI)	96.0
		Lactobacillus fermentum (RDP)	97.3

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
	T106	*Lactobacillus salivarius* (NCBI)	95.0
		Lactobacillus salivarius (RDP)	99.0
39	T107	*Lactobacillus mucosae* (NCBI)	99.0
(179)		*Lactobacillus mucosae* (RDP)	98.0
40	T109	*Lactobacillus fermentum* (NCBI)	96.0
(185)		*Lactobacillus fermentum* (RDP)	97.7
	T110	*Lactobacillus casei* (NCBI)	97.0
		Lactobacillus paracasei (NCBI)	97.0
		Lactobacillus rhamnosus (NCBI)	97.0
		Lactobacillus zeae (NCBI)	97.0
		Lactobacillus casei (RDP)	97.4
		Lactobacillus paracasei (RDP)	97.4
41	T113	*Lactobacillus salivarius* (NCBI)	95.0
(187)		*Lactobacillus salivarius* (RDP)	97.5
	XT114	Uncultured bacterium (NCBI)	98.0
		Bacterium ii1389 (RDP)	94.3
	XT118	*Lactobacillus gasseri* (NCBI)	89.0
	T117	Uncultured bacterium (NCBI)	94.0
		Lactobacillus agilis (NCBI)	95.0
		Lactobacillus salivarius (NCBI)	95.0
		Bacterium ii1389 (RDP)	98.0
42	T115	*Lactobacillus fermentum* (NCBI)	97.0
(190)		*Lactobacillus fermentum* (RDP)	97.9
	T116	*Lactobacillus salivarius* (NCBI)	96.0
		Lactobacillus salivarius (RDP)	100

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
43	T125	*Lactobacillus paracasei* (NCBI)	93.0
(193)		*Lactobacillus casei* (NCBI)	93.0
		Lactobacillus rhamnosus (NCBI)	93.0
		Lactobacillus casei (RDP)	95.0
		Lactobacillus paracasei (RDP)	95.0
		Lactobacillus rhamnosus (RDP)	95.0
(194)	T126	*Weissella cibaria* (NCBI)	95.0
		Weissella cibaria (RDP)	97.3
(195)	T127	*Weissella confusa* (NCBI)	95
		Weissella confusa (RDP)	96.7
44	T129	*Lactobacillus fermentum* (NCBI)	98.0
(200)		*Lactobacillus fermentum* (RDP)	98.7
	T130	*Lactobacillus fermentum* (NCBI)	98.0
		Lactobacillus fermentum (RDP)	98.6
	T131	*Lactobacillus casei* (NCBI)	99.0
		Lactobacillus rhamnosus (NCBI	99.0
		Lactobacillus paracasei (NCBI)	99.0
		Lactobacillus casei (RDP)	99.6
		Lactobacillus paracasei (RDP)	99.6
		Lactobacillus rhamnosus (RDP)	99.6
45	T134	*Lactobacillus salivarius* (NCBI)	97.0
(204)		*Lactobacillus salivarius* (RDP)	97.5
	T135	*Lactobacillus fermentum* (NCBI)	96.0
		Lactobacillus fermentum (RDP)	98.3
46	T137	*Lactobacillus fermentum* (NCBI)	97.0
(207)		*Lactobacillus fermentum* (RDP)	98.5

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
	T138	Uncultured bacterium (NCBI)	97.0
		Lactobacillus agilis (NCBI)	95.0
		Bacterium ii 1389 (RDP)	91.1
47	T139	*Lactobacillus fermentum* (NCBI)	98.0
(208)		*Lactobacillus fermentum* (RDP)	98.9
	T140	*Lactobacillus casei* (NCBI)	98.0
		Lactobacillus paracasei (NCBI)	98.0
		Lactobacillus casei (RDP)	98.9
		Lactobacillus paracasei (RDP)	98.9
		Lactobacillus rhamnosus (RDP)	98.9
48	XT146	*Lactobacillus gasseri* (NCBI)	98.0
(218)		*Lactobacillus gasseri* (RDP)	97.7
	T151	*Lactobacillus pontis* (NCBI)	97.0
		Lactobacillus pontis (RDP)	97.1
49	T156	*Lactobacillus salivarius* (NCBI)	95.0
(232)		*Lactobacillus salivarius* (RDP)	98.3
	T157	*Lactobacillus mucosae* (NCBI)	98.0
		Lactobacillus mucosae (RDP)	99.3
50	T161	*Lactobacillus panis* (NCBI)	96.0
(242)		*Lactobacillus panis* (RDP)	96.9
	T162	*Lactobacillus salivarius* (NCBI)	97.0
		Lactobacillus salivarius (RDP)	98.0
51	T168	*Lactobacillus salivarius* (NCBI)	97.0
(245)		*Lactobacillus salivarius* (RDP)	99.6

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	*Lactobacillus* isolate	Match organism	% Similarity
52	T169/1	*Lactobacillus casei* (NCBI)	96.0
(241)		*Lactobacillus paracasei* (NCBI)	96.0
		Lactobacillus zeae (NCBI)	96.0
		Lactobacillus rhamnosus (NCBI)	96.0
		Lactobacillus casei (RDP)	97.9
		Lactobacillus paracasei (RDP)	97.9
		Lactobacillus rhamnosus (RDP)	97.9
	T169/2	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	98.0
53	T171	*Lactobacillus salivarius* (NCBI)	97.0
(249)		*Lactobacillus salivarius* (RDP)	99.7
	T174	*Lactobacillus mucosae* (NCBI)	97.0
		Lactobacillus mucosae (RDP)	99.0
	T170	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	99.7
54	T172	*Lactobacillus salivarius* (NCBI)	96.0
(254)		*Lactobacillus salivarius* (RDP)	97.5
	T175	*Lactobacillus oris* (NCBI)	98.0
		Lactobacillus oris (RDP)	99.9
55	T176	*Lactobacillus casei* (NCBI)	97.0
		Lactobacillus paracasei (NCBI)	97.0
		Lactobacillus rhamnosus (NCBI)	97.0
		Lactobacillus casei (RDP)	100
		Lactobacillus paracasei (RDP)	100
56	XT179	*Lactobacillus gasseri* (NCBI)	96.0
(259)		*Lactobacillus gasseri* (RDP)	99.7

Table 13. Genotypic identification based on 16S r RNA gene sequencing of 92 *Lactobacillus* isolates from throat swabs of 57 syspeptic patients with severe gastritis. (Continued)

Subject and patient no.	***Lactobacillus* isolate**	**Match organism**	**% Similarity**
57	T184	*Lactobacillus salivarius* (NCBI)	98.0
(276)		*Lactobacillus salivarius* (RDP)	97.7
	T185	*Lactobacillus fermentum* (NCBI)	96
		Lactobacillus fermentum (RDP)	99.4

Table 14. Genotypic identification based on 16S rRNA gene sequencing of 32 *Lactobacillus* isolates from throat swabs of 21 syspeptic patients with peptic ulcer.

Subject and patient no.	*Lactobacillus* strain	Match organism of 16S rDNA	% Similarity
1	T8	*Lactobacillus fermentum* (NCBI)	98.0
(27)		*Lactobacillus fermentum* (RDP)	100
2	T9	*Lactobacillus fermentum* (NCBI)	98.0
(28)		*Lactobacillus fermentum* (RDP)	100
3	T10	*Lactobacillus salivarius* (NCBI)	99.0
(29)		*Lactobacillus salivarius* (RDP)	98.0
4	T22	*Lactobacillus fermentum* (NCBI)	96.0
(57)		*Lactobacillus fermentum* (RDP)	99.7
5	T24	*Lactobacillus fermentum* (NCBI)	94.0
(67)		*Lactobacillus fermentum* (RDP)	98.8
	T25	*Lactobacillus salivarius* (NCBI)	98.0
		Lactobacillus salivarius (RDP)	100
6	T30	*Lactobacillus salivarius* (NCBI)	99.0
(76)		*Lactobacillus salivarius* (RDP)	96.9
	T31	*Lactobacillus fermentum* (NCBI)	96.0
		Lactobacillus sp. (RDP)	99.7
		Lactobacillus fermentum (RDP)	99.7
7	T39	*Lactobacillus salivarius* (NCBI)	97.0
(93)		*Lactobacillus salivarius* (RDP)	98.9
8	T47	*Lactobacillus fermentum* (NCBI)	99.0
(99)		*Lactobacillus fermentum* (RDP)	97.6
9	T58	Bacterium ii1389 (NCBI)	97.0
(108)		Uncultured bacterium (NCBI)	97.0
		Bacterium ii1389 (RDP)	98.2
	T59	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	97.9

Table 14. Genotypic identification based on 16S rRNA gene sequencing of 32 *Lactobacillus* isolates from throat swabs of 21 syspeptic patients with peptic ulcer. (Continued)

Subject and patient no.	*Lactobacillus* strain	Match organism of 16S rDNA (forward sequence)	% Similarity
10 (109)	T60	*Lactobacillus paracasei* (NCBI	92.0
		Lactobacillus casei (NCBI)	92.0
		Lactobacillus casei (RDP)	97.8
		Lactobacillus paracasei (RDP)	97.8
	T63	*Lactobacillus mucosae* (NCBI)	95.0
		Lactobacillus mucosae (RDP)	99.1
11 (124)	T70	*Lactobacillus salivarius* (NCBI)	96.0
		Lactobacillus salivarius (RDP)	99.6
12 (127)	T71	*Lactobacillus salivarius* (NCBI)	99.0
		Lactobacillus salivarius (RDP)	99.2
	T72	*Lactobacillus fermentum* (NCBI)	98.0
		Lactobacillus fermentum (RDP)	99.5
13 (141)	T77	*Lactobacillus salivarius* (NCBI)	98.0
		Lactobacillus salivarius (RDP)	100
	T78	*Lactobacillus mucosae* (NCBI)	97.0
		Lactobacillus mucosae (RDP)	99.4
14 (156)	T88	*Lactobacillus murinus* (NCBI)	97.0
		Lactobacillus murinus (RDP)	98.6
15 (158)	T90	*Lactobacillus salivarius* (NCBI)	96.0
		Lactobacillus salivarius (RDP)	97.3
	T91	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	98.1
16 (170)	T102	*Lactobacillus mucosae* (NCBI)	98.0
		Lactobacillus mucosae (RDP)	99.3
	T103	*Lactobacillus pentosus* (NCBI)	98.0
		Lactobacillus plantarum (NCBI)	98.0
		Lactobacillus plantarum (RDP)	97.5

Table 14. Genotypic identification based on 16S r RNA gene sequencing of 32 *Lactobacillus* isolates from throat swabs of 21 syspeptic patients with peptic ulcer. (Continued)

Subject and patient no.	*Lactobacillus* strain	Match organism of 16S rDNA (forward sequence)	% Similarity
17 (182)	T108	*Lactobacillus casei* (NCBI)	97.0
		Lactobacillus rhamnosus (NCBI)	97.0
		Lactobacillus paracasei (NCBI)	97.0
		Lactobacillus zeae(NCBI)	97.0
		Lactobacillus casei (RDP)	98.5
		Lactobacillus paracasei (RDP)	98.5
	T111	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	98.4
18 (223)	T147	*Lactobacillus fermentum* (NCBI)	95.0
		Lactobacillus fermentum (RDP)	97.5
	T148	*Lactobacillus fermentum* (NCBI)	98.0
		Lactobacillus fermentum (RDP)	100
19 (234)	T158	*Lactobacillus salivarius* (NCBI)	96.0
		Lactobacillus salivarius (RDP)	98.7
	T159	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	99.3
20	T173	*Lactobacillus fermentum* (NCBI)	97.0
		Lactobacillus fermentum (RDP)	99.5
21 (257)	T177	*Lactobacillus fermentum* (NCBI)	98.0
		Lactobacillus fermentum (RDP)	97.7
	T178	*Lactobacillus casei* (NCBI)	97.0
		Lactobacillus paracasei (NCBI)	97.0
		Lactobacillus zeae (NCBI)	97.0
		Lactobacillus rhamnosus (NCBI)	97.0
		Lactobacillus casei (RDP)	99.6
		Lactobacillus paracasei (RDP)	99.6
		Lactobacillus rhamnosus (RDP)	99.6

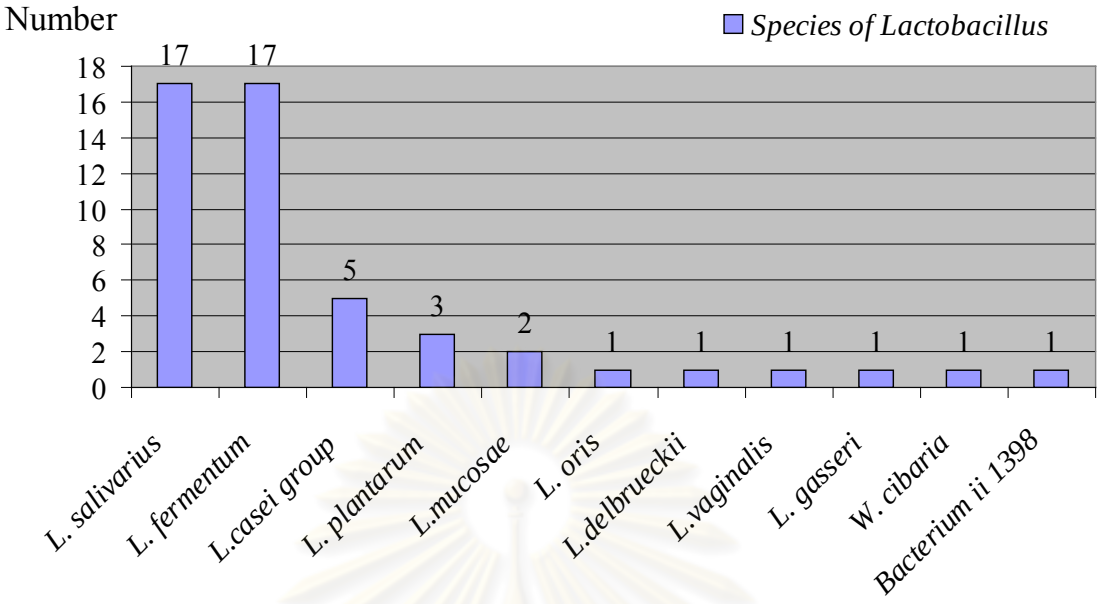


Figure 25. The species of *Lactobacillus* isolates from throat swabs of mild gastritis patients as identified by PCR and DNA sequencing.

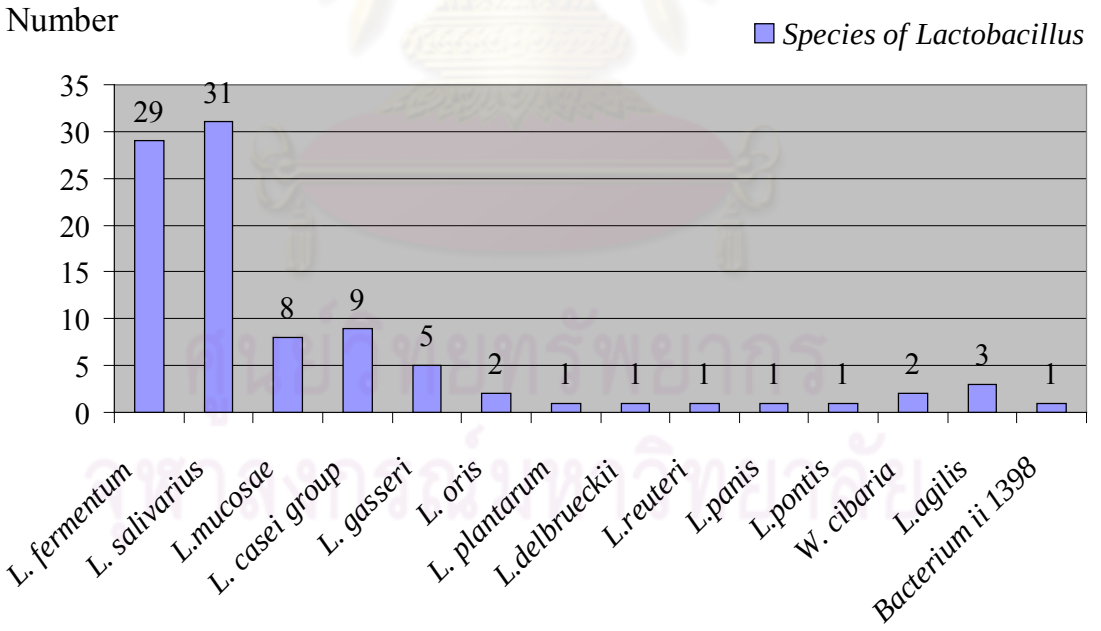


Figure 26. The species of *Lactobacillus* isolates from throat swabs of severe gastritis patients as identified by PCR and DNA sequencing.

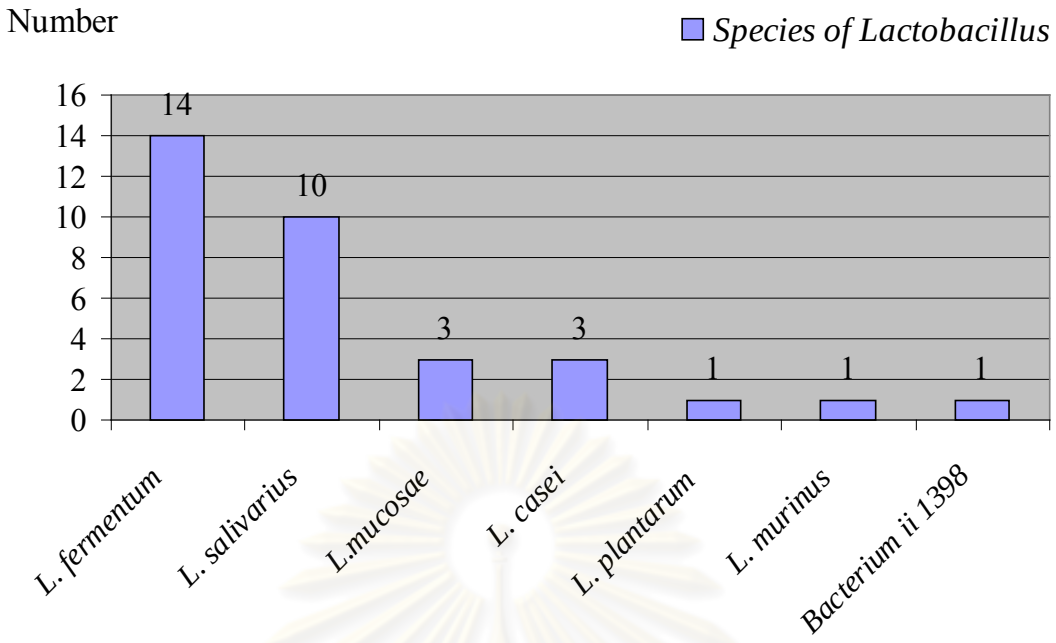


Figure 27. The species of *Lactobacillus* isolates from throat swabs of peptic ulcer patients as identified by PCR and DNA sequencing.

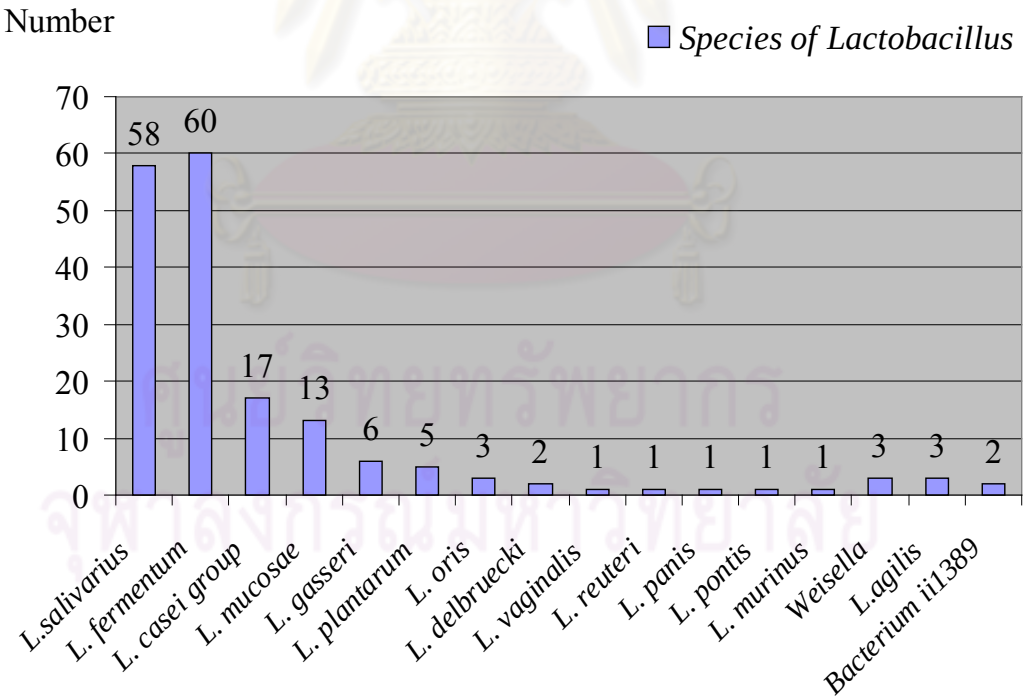


Figure 28. The species of bacterial isolates from throat swabs of dyspeptic patients as identified by PCR and DNA sequencing.

3. Comparison of the presence of *Lactobacillus* in each group of patients

From the results of genotypic identification, the number of dyspeptic patients from whom *Lactobacillus* isolates in gastric biopsies were recovered and the number of *Lactobacillus* isolates from these patients were shown in Table 15. The number of dyspeptic patients from whom *Lactobacillus* in gastric biopsies were recovered, when compared between group 1 with group 2, was not significantly different (p-value >0.05). While the comparison between group 2 with group 3 and group 1 with group 3 showed the significantly different results at p-value <0.05 and <0.01, respectively (Table 16).

In addition, the number of dyspeptic patients from whom *Lactobacillus* isolates in throat swabs were recovered and the number of *Lactobacillus* isolates from these patients were shown in Table 17. The number of dyspeptic patients from whom *Lactobacillus* in throat swabs were recovered, when compared among these 3 groups, were not significantly different (p-value >0.05) (Table 18).

Table 15. The number of dyspeptic patients from whom *Lactobacillus* isolates in gastric biopsies were recovered and the number of *Lactobacillus* isolates from these patients.

Group of patient	**Total patients**	**Patients with *Lactobacillus* (%)**	**Number of *Lactobacillus* isolate (%)**
Group 1	70	9 (12.85)	16 (22.86)
Group 2	158	32 (20.25)	47 (29.75)
Group 3	44	16 (36.36)	24 (54.55)
Total	272	57 (20.96)	87 (31.99)

Table 16. The comparison of number of dyspeptic patients from whom *Lactobacillus* isolates in gastric biopsies were recovered. Statistical values were calculated using the Chi square which was considered statistically significant at $p \leq 0.05$.

Group of patient	**The number of patients with *Lactobacillus***
Group 1 : Group 2	Not significantly different
Group 2 : Group 3	Significantly different ($p < 0.05$)
Group 1 : Group 3	Significantly different ($p < 0.01$)

Table 17. The number of dyspeptic patients from whom *Lactobacillus* isolates in throat swabs were recovered and the number of *Lactobacillus* isolates from these patients.

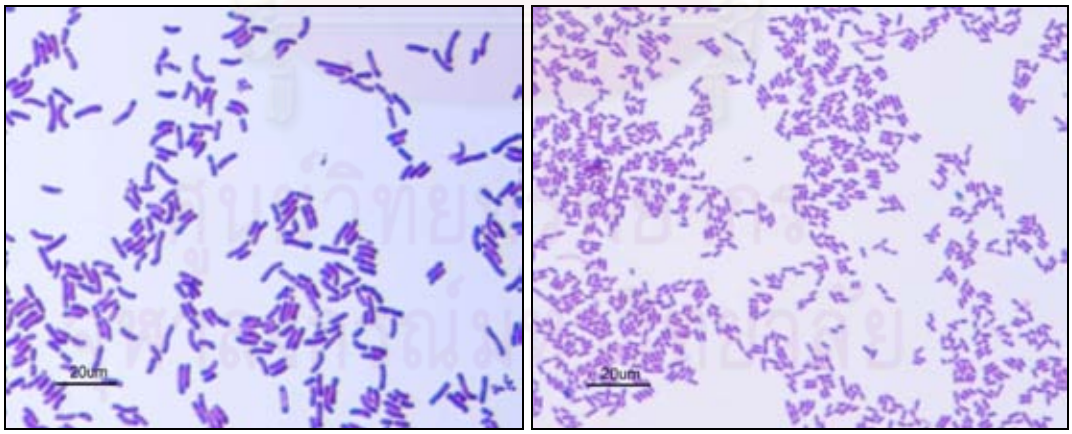
Group of patient	**Total patients**	**Patients with *Lactobacillus* (%)**	**Number of *Lactobacillus* isolate (%)**
Group 1	70	25 (35.71)	48 (68.57)
Group 2	158	57 (36.08)	92 (58.23)
Group 3	44	21 (47.73)	32 (72.73)
Total	272	103 (37.87)	172 (63.24)

Table 18. The comparison of number of dyspeptic patients from whom *Lactobacillus* isolates in throat swab were recovered. Statistical values were calculated using the Chi square which was considered statistically significant at $p \leq 0.05$.

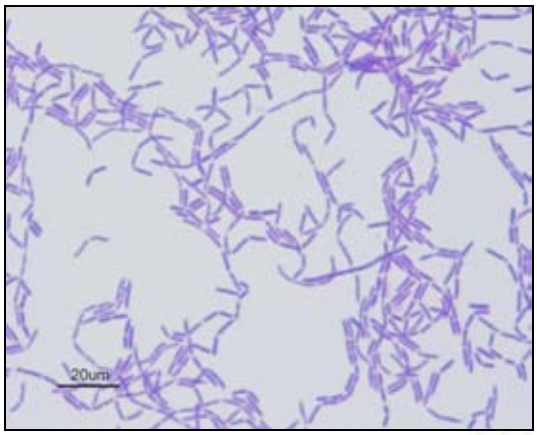
Group of patient	**The number of patients with *Lactobacillus***
Group 1 : Group 2	Not significantly different
Group 2 : Group 3	Not significantly different
Group 1: Group 3	Not significantly different

4. Cell morphology of *Lactobacillus* isolates from gastric biopsies and throat swabs of dyspeptic patients

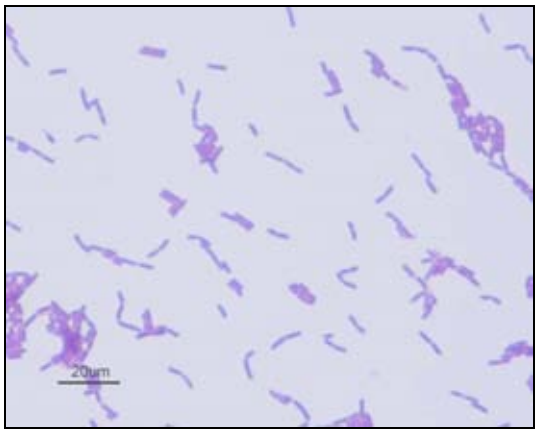
The morphology of *Lactobacillus* cells were demonstrated by gram staining. gram stain was performed to observe microscopic morphologies of *Lactobacillus* isolated from gastric biopsies and throat swabs of dyspeptic patients. They were gram-positive rod, non-spore forming rods or coccobacilli or long and slender rod. Some species exhibited bipolar bodies or internal granulation. *L. salivarius* was gram-positive straight rod and with rounded ends, occuring in single and in pair and internal granulation. *L. fermentum* was gram-positive short rod, occuring in single and in pair. *L. gasseri* was gram-positive long rod, occuring in pair and in chains. *L. mucosa* was gram-positive rod and occuring in pairs. *L. murinus* was gram-positive, slender or straigth rods and with rounded ends, arrangement in single and in pairs. *L. oris* was short rods and occuring in single, in pair and in chains. *L. plantarum* was gram-positive, straigth rods and occuring in single. *L. casei* was gram-positive rod and occuing in single and in pair. *L. reuteri* was slightly irregular rods or bent rod into coccobacilli and were difficult separated from gram-positive cocci. *L. delbrueckii* was gram-positive long rod with rounded ends and occuring in pair and in short chains. Figures 29 and 30 demonstrated the morphologies of *Lactobacillus* isolates from gastric biopsies and throat swabs, respectively.



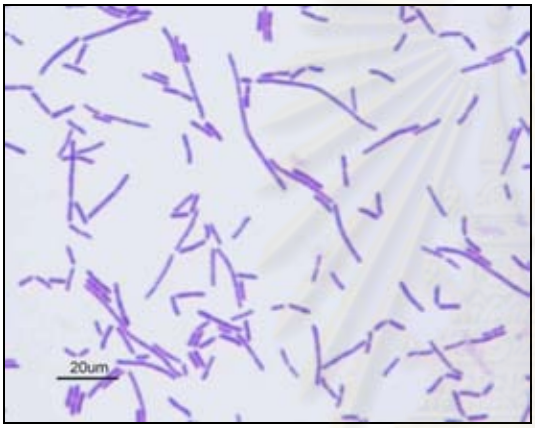
B101 *Lactobacillus salivarius* B84 *Lactobacillus fermentum*



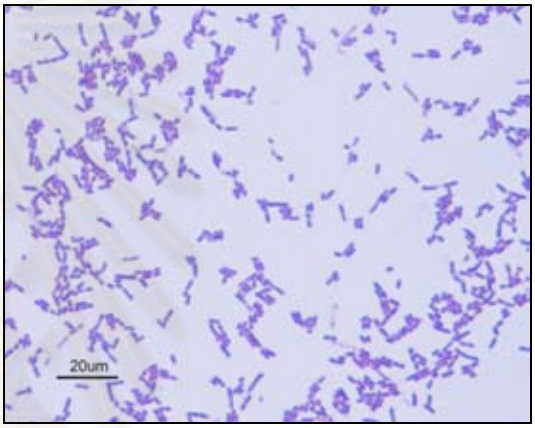
XB48 *Lactobacillus gasseri*



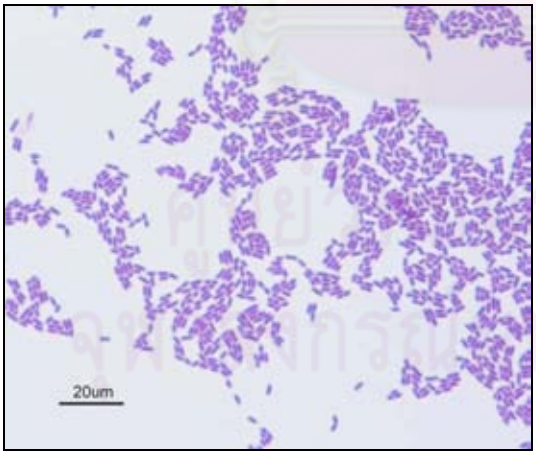
B79 *Lactobacillus mucosae*



B57 *Lactobacillus murinus*



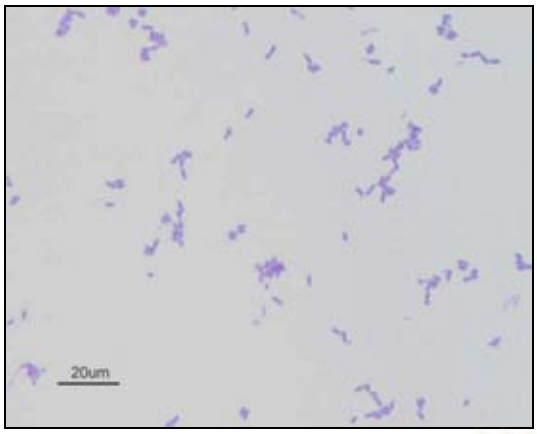
B93 *Lactobacillus oris*



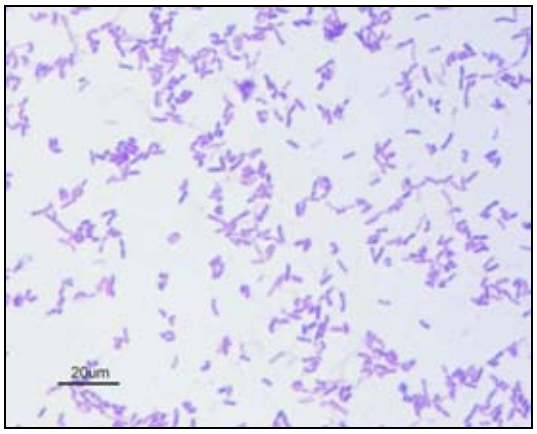
B87 *Lactobacillus plantarum*

B59 *Weissella confuse*

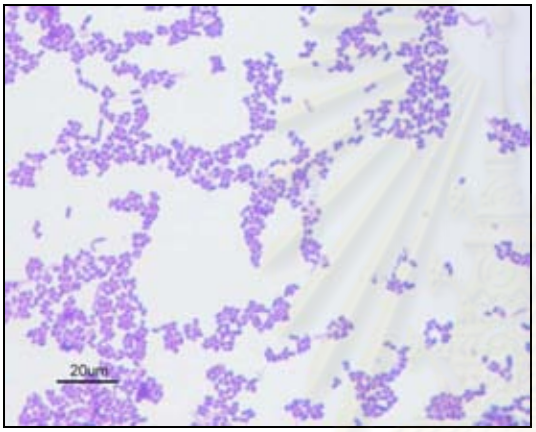
Figure 29. Cell morphology of *Lactobacillus* isolates from gastric biopsies of dyspeptic patients



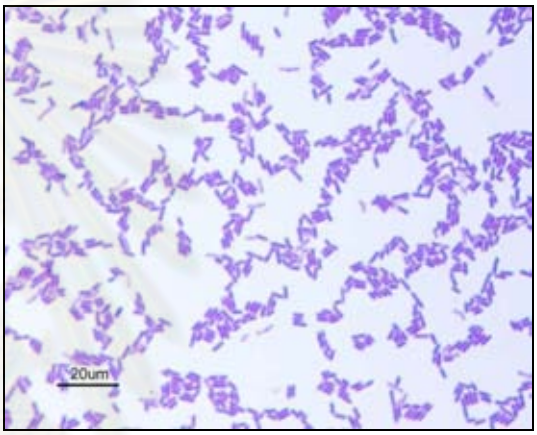
T31 *Lactobacillus fermentum*



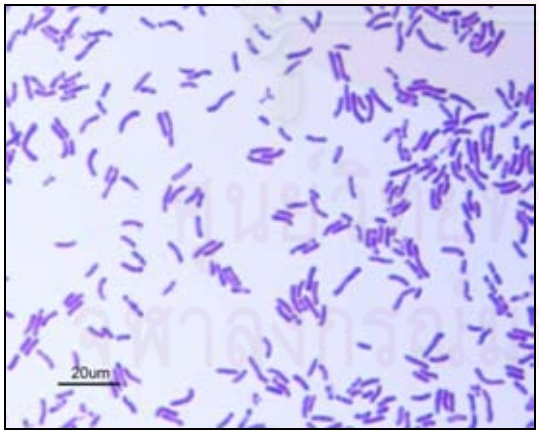
T32/1 *Lactobacillus casei*



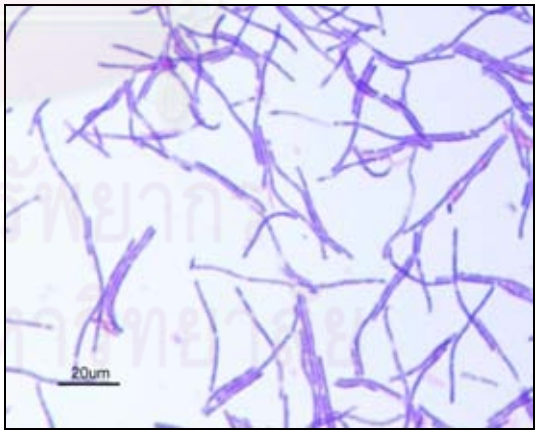
T36 *Lactobacillus reuteri*



T181 *Lactobacillus fermentum*



T182 *Lactobacillus salivarius*



T183 *Lactobacillus delbrueckii*

Figure 30. Cell morphology of *Lactobacillus* isolates from throat swabs of dyspeptic patients.

5. Comparison of *Lactobacillus* species isolated from gastric biopsies and throat swabs

It was found that a total of 126 patients from whom *Lactobacillus* spp. were detected in either gastric biopsies alone (19 subjects) or throats alone (69 subjects) and both in gastric biopsies and throats (38 subjects). Species of *Lactobacillus* detected in both gastric biopsies and throats were reviewed in each patient. Of 38 patients, 28 (73.68%) had at least one isolate belonging to the same species. When categorized in group of patient, there were 6 of 7, 13 of 20 and 9 of 11 subjects in groups 1, 2 and 3 patients, respectively (Tables 19, 20 and 21). The species that were detected most were *L. fermentum* and *L.salivarius.*

The species that were found in each group of patients and all patients were shown in Tables 22 and 23, respectively.The species that were predominate in gastric biopsies were *L. fermentum, L. salivarius* and *L. gasseri* whereas the predominant species found in throats were *L. fermentum, L. salivarius, L. casei* group and *L. mucosae* (Table 23). Various species of *Lactobacillus* were isolated more from throat swabs than gastric biopsies (Table 23).

Table 19. Comparison of *Lactobacillus* species isolated from gastric biopsies and throat swabs of mild gastritis patients. Species isolated from both gastric biopsy and throat swab were highlighted.

Subject and patient no.	**Isolates from gastric biopsies**		**Isolates from throat swabs**	
	Code	***Lactobacillus* species**	**Code**	***Lactobacillus* species**
1. (56)	B13	*L. casei* group	T21	*L. casei* group
2. (163)	B66 XB68	*L. fermentum* *L. gasseri*	T97 T95 T96	*L. fermentum* *L. mucosae* *L. salivarius*
3. (225)	B91 B92 B94	*L. salivarius* *L. fermentum* *L. gasseri*	T152 T153	*L. salivarius* *L. fermentum*
4. (227)	B93	*L. oris*	T154 T155	*L. salivarius* *L. vaginalis*
5. (267)	B101	*L. salivarius*	T181 T182 T183	*L. fermentum* *L. salivarius* *L. delbrueckii*
6. (286)	B105 B106 B107	*L. fermentum* *L. casei* group *L. casei* group	T186 T187 T188	*L. fermentum* *L. casei* group *L. casei* group
7. (292)	B108 B109 B110	*L. fermentum* *L. salivarius* *L. salivarius*	T192 T193 T194	*L. fermentum* *L. salivarius* *L. salivarius*

Table 20. Comparison of *Lactobacillus* species isolated from gastric biopsies and throat swabs of severe gastritis patients. Species isolated from both gastric biopsy and throat swab were highlighted.

Subject and patient no.	Isolates from gastric biopsies		Isolates from throat swabs	
	Code	*Lactobacillus* species	Code	*Lactobacillus* species
1. (26)	B2	*L. fermentum*	T5 T6	*L. fermentum* *L. salivarius*
2. (44)	B8	*L. salivarius*	T18	*L. salivarius*
3. (68)	B18 XB19	*L. oris* *L.gasseri*	T27	*L. oris*
4. (70)	B20	*L. fermentum*	T26	*L.salivarius*
5. (94)	B29	*L. fermentum*	T40 T41	*L. salivarius* *L. fermentum*
6. (95)	B31	*L. fermentum*	T42	*L. salivarius*
7. (96)	XB30 B35	*L. gasseri* *L. fermentum*	T43 XT44/1 T44/2	*L. salivarius* *L. fermentum* *L. delbrueckii*
8. (105)	XB41	*L. gasseri*	T57 T64	*L. fermentum* *L. fermentum*
9. (120)	B42	*L fermentum*	T66	*L. fermentum*
10. (121)	XB45	*L. gasseri*	T67 T68	*L. salivarius* *L. salivarius*
11. (137)	XB49	*L. gasseri*	T76	*L. salivarius*
12. (153)	B53 B54	*L. salivarius* *L.mucosae*	T83 T84	*L. salivarius* *L.mucosae*
13. (154)	XB58 B64 B60	*L. gasseri* *L. plantarum* *L. salivarius*	T86 T87	*L.salivarius* *L. fermentum*

Table 20. Comparison of *Lactobacillus* species isolated from gastric biopsies and throat swabs of severe gastritis patients. Species isolated from both gastric biopsy and throat swab were highlighted. (Continued)

Subject and patient no.	**Isolates from gastric biopsies**		**Isolates from throat swabs**	
	Code	***Lactobacillus* species**	**Code**	***Lactobacillus* species**
14. (155)	B55	*L. salivarius*	T85	*L. salivarius*
15. (185)	B72 B73	*L. fermentum* *L. salivarius*	T109 T110	*L. fermentum* *L. casei group*
16. (187)	B76	*L. fermentum*	T113 XT114 T117 XT118	*L. salivarius* *uncultured bacterium* or Bacterium ii 1398 Bacterium ii1389 *L. gasseri*
17.(190)	B74 XB75 XB77 B78	*L. salivarius* *L.fermentum* *L. gasseri* *L. salivarius*	T115 T116	*L. fermentum* *L. salivarius*
18. (200)	B82 B83	*L. fermentum* *L. fermentum*	T129 T130 T131	*L. fermentum* *L. fermentum* *L. casei*
19. (232)	XB96	*L. gasseri*	T156 T157	*L. salivarius* *L. mucosae*
20. (276)	B102 B103	*L. salivarius* *L. casei*	T184 T185	*L. salivarius* *L. fermentum*

Table 21. Comparison of *Lactobacillus* species isolated from gastric biopsies and throat swabs of peptic ulcer patients. Species isolated from both gastric biopsy and throat swab were highlighted.

Subject and patient no.	**Isolates from gastric biopsies**		**Isolates from throat swabs**	
	Code	***Lactobacillus* species**	**Code**	***Lactobacillus* species**
1. (27)	XB7	*L. plantarum*	T8	*L. fermentum*
2. (28)	B4/2 B5	*L. salivarius* *L. fermentum*	T9	*L. fermentum*
3. (57)	B14	*L. fermentum*	T22	*L. fermentum*
4. (67)	B15 B16	*L. mucosae* *L.salivarius*	T24 T25	*L. fermentum* *L. salivarius*
5. (76)	B23 B24	*L. salivarius* *L. fermentum*	T30 T31	*L. salivarius* *L. fermentum*
6. (99)	B32 B33	*L. salivarius* *L. fermentum*	T47	*L. fermentum*
7. (108)	B36 B37	*L. agilis* *L. salivarius*	T59	*L. salivarius*
8. (109)	XB40	*L. gasseri*	T60 T63	*L. casei* *L. mucosae*
9. (156)	B57	*L. murinus*	T88	*L. murinus*
10. (158)	B61 B62	*L. fermentum* *L. salivarius*	T90 T91	*L. salivarius* *L. fermentum*
11. (257)	B99	*L. fermentum*	T177 T178	*L. fermentum* *L. casei*

Table 22. Species and number of *Lactobacillus* isolated from gastric biopsies and throat swabs of each group of patients.

***Lactobacillus* species**	**Group 1**		**Group 2**		**Group 3**	
	Gastric biopsy (%)	**Throat swab (%)**	**Gastric biopsy (%)**	**Throat swab (%)**	**Gastric biopsy (%)**	**Throat swab (%)**
L. fermentum	5(31.25)	17(35.42)	15(31.91)	29(31.18)	9(37.50)	14(43.75)
L. salivarius	4(25)	17(35.42)	10(21.28)	31(33.33)	9(37.50)	10(31.25)
L. casei group	3(18.75)	5(10.42)	1(2.13)	9(9.68)	-	3(9.36)
L. mucosae	-	2(4.17)	3(6.38)	8(8.60)	2(8.33)	3(9.36)
L. gasseri	2(12.5)	1(2.08)	10(21.28)	5(5.38)	1(4.17)	-
L. plantarum	1(6.25)	3(6.25)	4(8.51)	1(1.08)	1(4.17)	1(3.13)
L. plantarum or *L. pentosus*	-	-	2(4.26)	-	-	-
L. vaginalis	-	1(2.08)	-	-	-	-
L. oris	1(6.25)	1(2.08)	2(4.26)	2(2.15)	-	-
L. delbrueckii	-	1(2.08)	-	1(1.08)	-	-
L.reuteri	-	-	-	1(1.08)	-	-
L.oris	-	-	-	1(1.08)	-	-
L.panis	-	-	-	1(1.08)	-	-
L. murinus	-	-	-	-	1(4.17)	1(3.13)
L.pontis	-	-	-	1(1.08)	-	-
Lastobacillus sp. 52A	-	-	-	-	1(4.17)	-
L. agilis	-	-	-	3(3.23)	-	-
Total	16	48	47	93	24	32

Table 23. Comparison of the number of *Lactobacillus* isolated from gastric biopsies and throat swabs of dyspeptic patients.

***Lactobacillus* species**	**No. of *Lactobacillus* isolates from gastric biopsy**	**No. of *Lactobacillus* isolates from throat swab**
L. fermentum	29(33.33)	60(34.88)
L. salivarius	23(26.44)	58(33.72)
L. casei group	4(4.60)	17(9.88)
L. mucosae	5(5.75)	13(7.56)
L. gasseri	13(14.94)	6(3.49)
L. plantarum	6(6.90)	5(2.91)
L. plantarum or *L. pentosus*	2(2.30)	-
L. oris	3(3.45)	3(1.74)
L. delbrueckii	-	2(1.16)
L.reuteri	-	1(0.58)
L.panis	-	1(0.58)
L.pontis	-	1(0.58)
L.vaginalis	-	1(0.58)
L. murinus	1(1.15)	1(0.58)
L. agilis	-	3(1.74)
Lactobacillus species 52A	1(1.15)	-
Total	87	172

6. Immunomodulatory effect of *Lactobacillus* isolates from gastric biopsies of dyspeptic patients on TNF-α production in LPS-activated THP-1 human monocytic cells

Eighty seven *Lactobacillus* isolates from gastric biopsies were recovered from -80°C and cultivated in MRS broth for 24 and 48 h for preparation of *Lactobacillus* conditioned media (LCM) to determine the modulation of TNF-α production in LPS-activated THP-1 human monocytic cells. The bioassay was performed by using THP-1 cells incubated with LCM of each isolate of *Lactobacillus* and activated with LPS of *E. coli* serotype O 127:B8 for induction TNF-α production as described in materials and methods. The supernatant containing secreted TNF-α was collected and measured by using cytokine-specific sandwich quantitative ELISA. The concentration of TNF-α was quantified by using standard curve of recombinant human TNF-α and calculated by using Microsoft excel based on linear relationship as $y = mx + b$. A value of $R^2 = 1$ indicated an exact linear relationship between x and y as shown in Figure 36. Percentage of TNF-α inhibition and cell viability were calculated by the formula as follows.

$$\% \text{ TNF-}\alpha \text{ inhibition} = \left((O/B)-1 \right) \times 100$$

O = observed, secreted TNF-α of experiment (pg/ml)

B = baseline, secreted TNF-α of MRS bacterial media control (pg/ml)

$$\% \text{ cell viability} = \left(\left(\frac{\text{dead cell}}{\text{total cell}} \right) - 1 \right) \times 100$$

Accepted cell viability as ≥ 80%

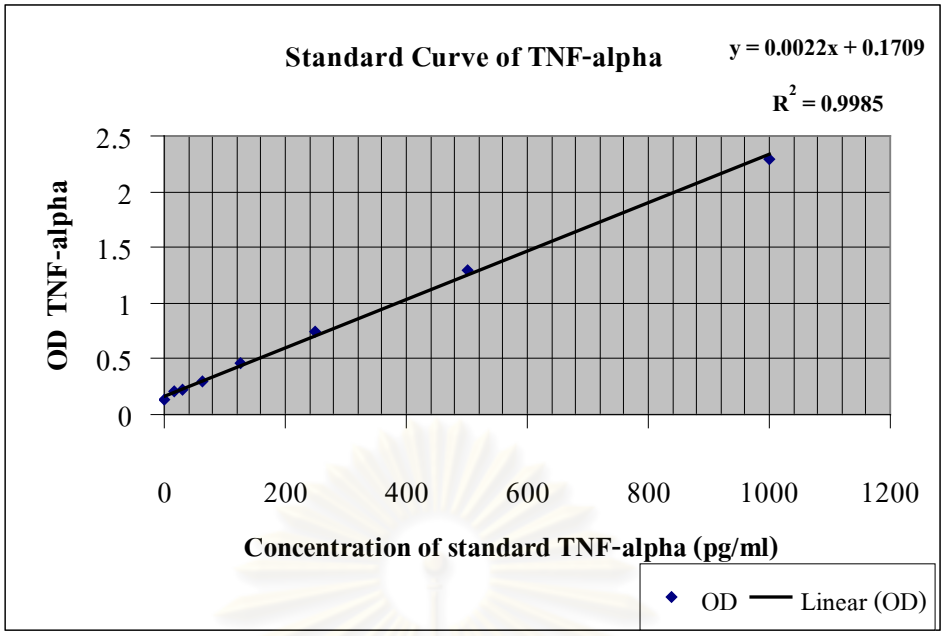


Figure 31. Standard curve of TNF-α determination at the concentration 15.625, 31.5, 62.5, 125, 250, 500, and 1000 pg/ml. $y = mx + b$, linear relationship; R^2=1.

The immunomodulatory activities of *Lactobacillus* isolates from group 1 patients with mild gastritis, group 2 patients with severe gastritis and group 3 patients with peptic ulcer were shown in Tables 24-31 and Figures 32-39, Tables 34-57 and Figures 42-65 and Tables 60-71 and Figures 68-79, respectively. These Tables and figures showed the level of TNF-α secretion by THP-1 monocytic cells when incubated with *Lactobacillus* conditioned media (LCM) of each *Lactobacillus* isolate (prepared at 24 and 48 h) in the presence or absence of LPS. The level of TNF-α was indicated with mean and standard deviation (SD) in tables and figures. The percentage of TNF-α inhibition was exhibited as (-) in the table. L58/1, TNF-α inhibitory strain used as positive control, suppressed TNF-α production and did not activate TNF-α in the absence of LPS. L9/7, non-TNF-α inhibitory strain used as negative control, did not suppress TNF-α production and this strain stimulated TNF-α production without LPS activation. MRS media control had no effect on TNF-α production. LPS stimulated TNF-α production on THP-1 monocytic cells as shown in the right of each figure. In the presence of LCM, TNF-α production was suppressed in various magnitude among *Lactobacillus* isolates. LCM of most isolates did not activate TNF-α production in the absence LPS, while LCM of some isolates stimulated TNF-α production by themselves.

The summary of immunomodulatory effects of *Lactobacillus* isolates from gastric biopsies of group 1 patients with mild gastritis, group 2 patients with severe gastritis and group 3 patients with peptic ulcer was shown in Table 32 and Figures 40-41, Table 58 and Figures 66-67 and Table 72 and Figures 80-81, respectively. Species of these *Lactobacillus* isolates were shown in Tables 33, 59 and 73.

From 87 isolates, 38 (43.68%) significantly suppressed TNF-α production. These TNF-α inhibitory isolates were 10 of 16, 22 of 47 and 6 of 24 isolates from patients with mild gastritis, severe gastritis and peptic ulcer, respectively.(Table 74)

The prevalence of *Lactobacillus* that significantly inhibited TNF-α in each group of patients were compared using chi square test which was considered statistically significant at p-value ≤ 0.05. As shown in Table 75, the prevalence of TNF-α- inhibitory *Lactobacillus* in patients groups 1 and 2, and groups 2 and 3 were not significantly different ($p>0.05$) but significantly different in patients groups 1 and 3 ($p=0.053$). Multivariate analysis of these data was performed and it was found that the prevalence of TNF-α- inhibitory *Lactobacillus* in patients groups 1 and 3 was not significantly different ($p=0.985$).

Table 24. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	735.74±11.18		0.00	858.7±83.51	
MRS	0.00	560.93±46.14	-23.76	0.00	601.3±52.70	-29.98
L58/1	0.00	313.89±60.69	-44.04	0.00	337.96±21.78	-43.79
L 9/7	463.52±13.98	545±20.76	-2.84	469.81±13.26	656.11±86.60	9.12
B13	0.00	402.41±46.03	-28.26	27.59±1.70	502.41±48.13	-16.45
B25	309.44±17.25	580.93±18.10	3.57	344.26±18.90	617.59±51.09	2.71
B66	278.7±24.65	643.15±25.27	14.66	317.59±16.38	700.19±25.64	16.45

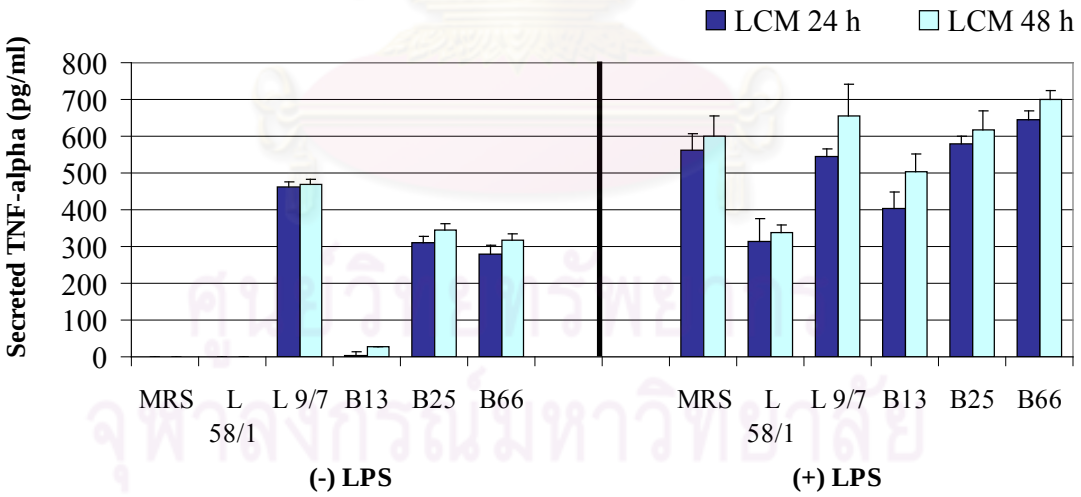


Figure 32. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 25. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1818.67±307.25		0.00	1459.14±188.32	
MRS	0.00	992.48±180.82	-45.43	0.00	910.57±84.02	-37.60
L58/1	0.00	616.29±127.43	-37.90	0.00	437.24±146.01	-51.98
L 9/7	799.14±94.49	1060.57±54.27	6.86	875.81±55	971.52±47.84	6.69
XB68	0.00	474.38±80.12	-52.20	0.00	331.05±82.13	-63.64
B92	470.1±121.0	828.19±34.33	-16.55	525.33±17.75	1161.52±241.49	27.56

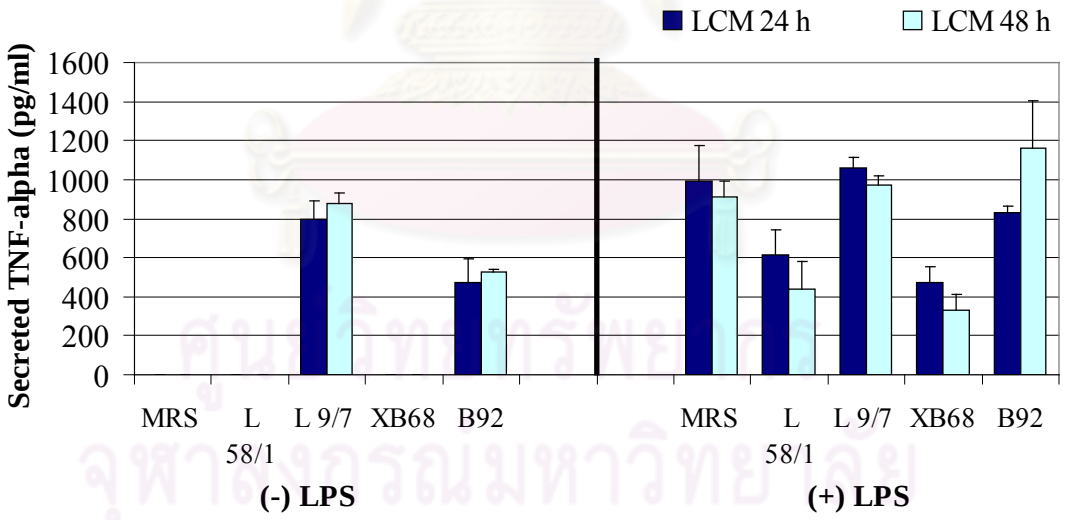


Figure 33. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 26. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1257.85±189.29		0.00	1347.08±50.04	
MRS	0.00	1267.85±216.48	0.80	0.00	1049.38±57.66	-22.10
L 58/1	0.00	759.38±54.39	-40.10	0.00	592.46±153.39	-43.54
L 9/7	905.54±56.57	1204.77±342.67	-4.98	915.54±5.44	1162.46±17.41	10.78
B90	0.00	667.85±133.81	-47.32	0.00	866.31±55.48	-17.45
B91	614.00±190.37	811.69±217.57	-35.98	353.23±15.23	431.69±43.51	-58.86

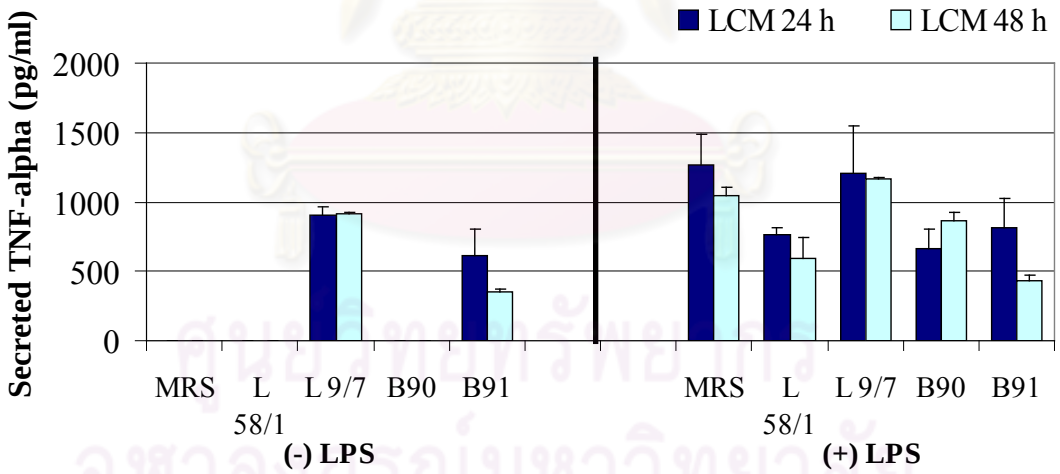


Figure 34. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 27. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1105.48±60.25		0.00	1199.56±216.97	
MRS	0.00	866.59±16.19	-21.61	0.00	904.74±29.19	-24.58
L58/1	0.00	361.04±25.05	-58.34	0.00	376.96±58.89	-58.33
L 9/7	626.22±75.29	815.85±23.99	-5.86	583.26±41.06	778.44±80.56	-13.96
B93	549.93±46.03	862.52±87.22	0	551.41±26.67	827.33±20.76	-8.56

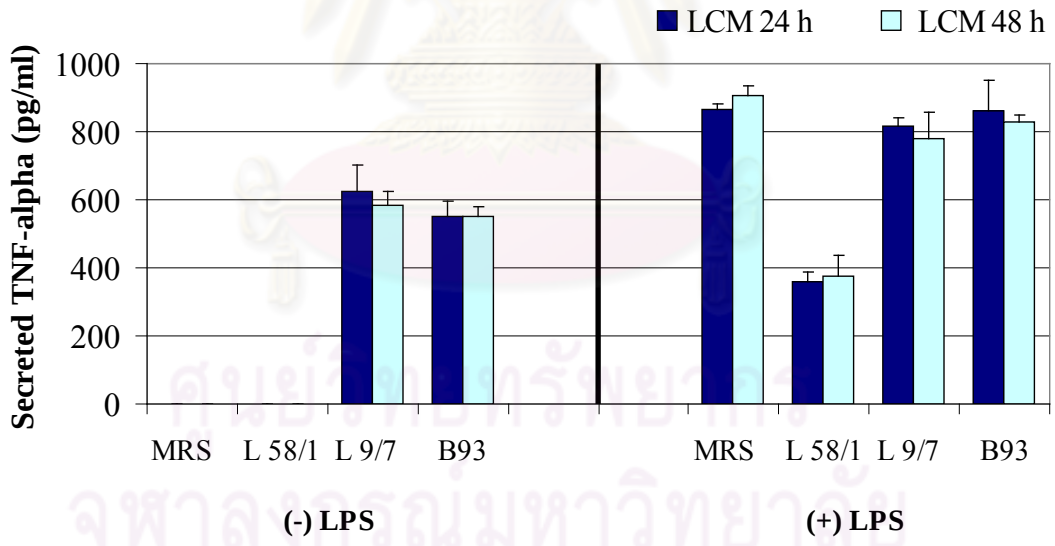


Figure 35. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 28. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1339.14±29.1		0.00	1304.21±97.31	
MRS	0.00	985.81±123.80	-26.38	0.00	921.22±101.96	-29.37
L58/1	0.00	621.90±62.32	-36.91	0.00	366.74±39.44	-60.19
L 9/7	774.09±60.60	1086.04±88.80	10.17	882.83±75.08	1093.63±101.30	18.72
XB94	3.29±4.38	740.75±80.38	-24.86	0.00	662.83±83.72	-28.05
B101	48.11±16.53	604.43±179.41	-38.69	201.91±14.89	876.62±111.81	-4.84
B106	0.00	473.86±126.0	-51.93	0.00	598.00±179.26	-35.09

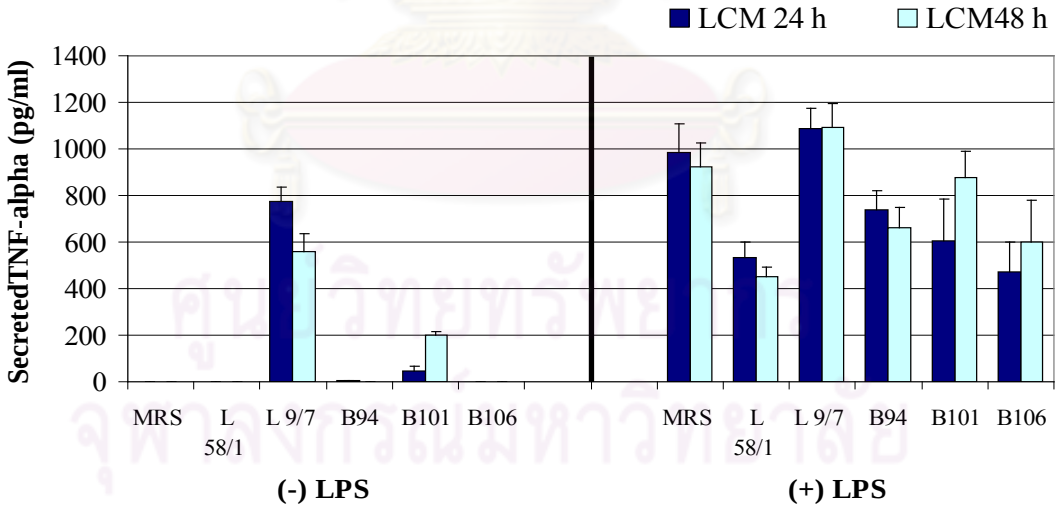


Figure 36. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 29. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	16.56±9.62	840.26±83.89		15.85±18.75	723.22±44.94	
MRS	0.00	637.67±17.36	-24.11	37.67±16.37	548.41±36.15	-24.17
L 58/1	0.00	428.78±64.47	-32.76	0.00	288.41±101.33	-47.41
L 9/7	473.59±9.56	632.48±111.98	0	462.48±4.49	545.81±7.06	0
B105	266.93±24.38	661.37±35.07	3.72	258.78±20.09	605.07±87.32	10.33

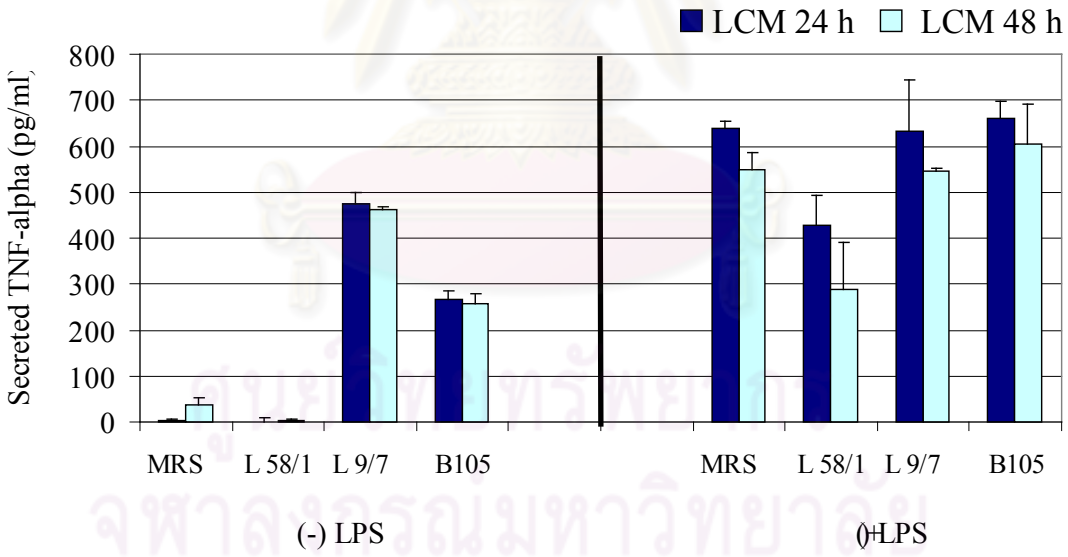


Figure 37. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 30. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1982.29±35.24		0.00	2135.91±134.34	
MRS	0.00	1592.14±52.58	-19.68	0.00	1454.17±178.67	-31.92
L58/1	0.00	971.28±160.92	-39.00	0.00	872.14±35.18	-40.02
L9/7	1438.52±82.42	1729.25±66.86	8.61	1544.03±59.88	1763.16±35.54	21.25
B107	0.00	1409.25±79.80	-11.49	0.00	955.91±62.08	-34.26
B109	346.93±14.97	1331.86±90.14	-16.35	585.77±15.66	1190.41±225.33	-18.14

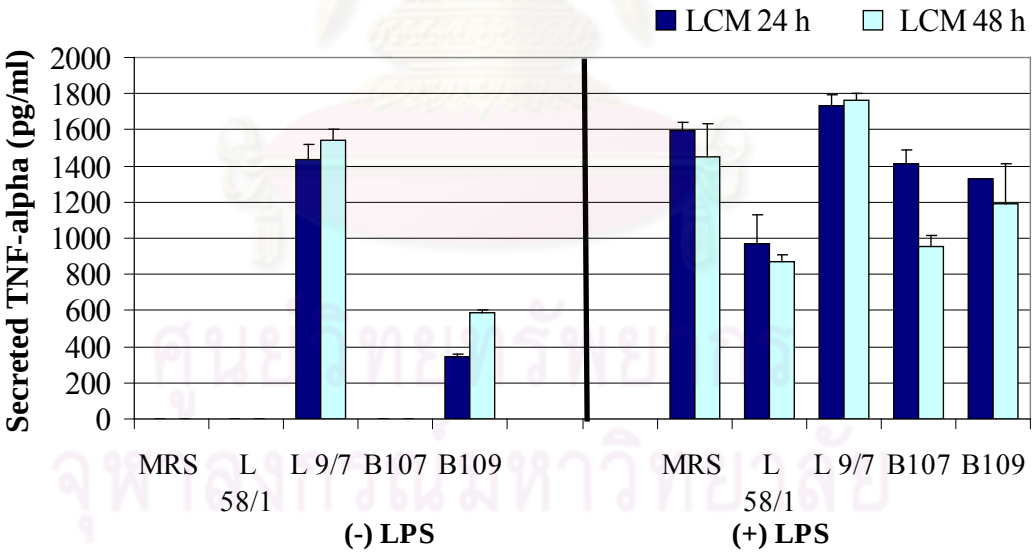


Figure 38. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 31. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	2173.86±123.24		0.00	2351.95±152.36	
MRS	0.00	1586.71±214.15	-27.01	0.00	1757.19±87.51	-25.29
L 58/1	36.71±24.37	1600.29±81.82	0	36.71±25.52	1025.29±47.72	-41.65
L 9/7	1573.86±53.70	1641.71±31.31	3.47	1573.86±76.59	1832.9±201.68	4.31
B108	464.81±33.11	1758.86±45.46	10.85	464.81±92.92	1902.9±67.92	8.29
B110	967.67±157.25	1619.57±264.66	2.07	967.67±94.78	950.52±241.10	-45.91

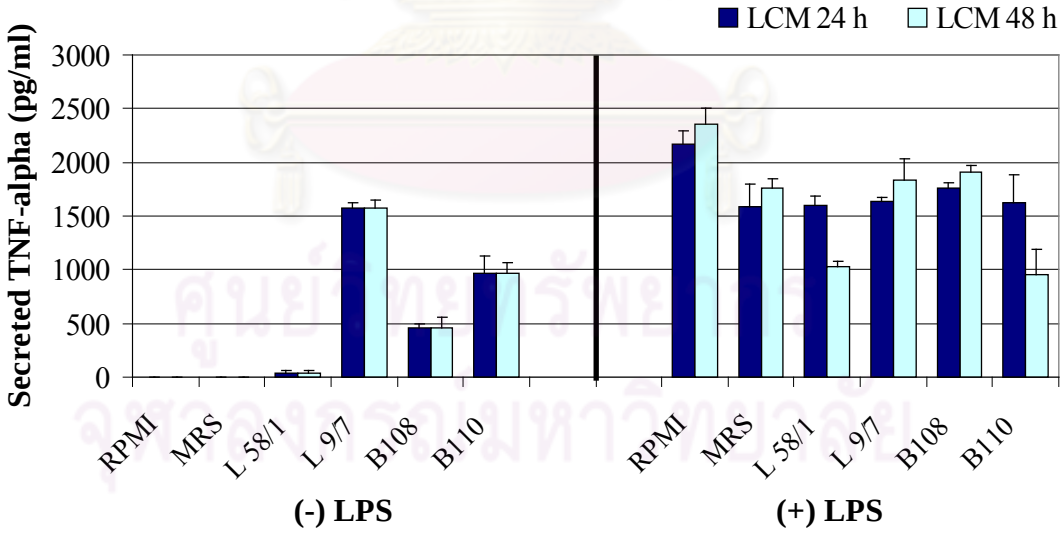


Figure 39. Immunomodulatory effects of *Lactobacillus* isolates from group 1 patients with mild gastritis patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 32. Summary of immunomodulatory effects of 16 *Lactobacillus* isolates from group 1 patients with mild gastritis (9 subjects) on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; % inhibition , - indicated inhibition.

Subject And Patient	**LCM+LPS 24h**	**% TNF-α inhibition**	**P-value**	**LCM+LPS 48h**	**% TNF-α inhibition**	**P-value**
Positive control	L 58/1	-36.12	<0.05	L 58/1	-48.36	<0.05
Negative control	L 9/7	1.06	-	L 9/7	3.76	-
1 (56)	B13	-28.26	<0.05	B13	-16.45	<0.05
2 (77)	B25	3.57	-	B25	2.71	-
3 (163)	B66 XB68	14.66 -52.2	- <0.05	B66 XB68	16.45 -63.64	- <0.001
4 (217)	B90	-47.32	<0.05	B90	-17.45	<0.05
5 (225)	B91 B92 XB94	-35.98 -16.55 -24.86	- - <0.05	B91 B92 XB94	-58.86 27.56 -28.05	<0.05 - <0.05
6 (227)	B93	0	-	B93	-8.56	-
7 (267)	B101	-38.69	<0.05	B101	-4.84	-
8 (286)	B105 B106 B107	3.72 -51.93 -11.49	- <0.005 <0.05	B105 B106 B107	10.33 -35.09 -34.26	- <0.05 <0.01
9 (292)	B108 B109 B110	10.85 -16.35 2.07	- <0.01 -	B108 B109 B110	8.29 -18.14 -45.91	- - <0.05

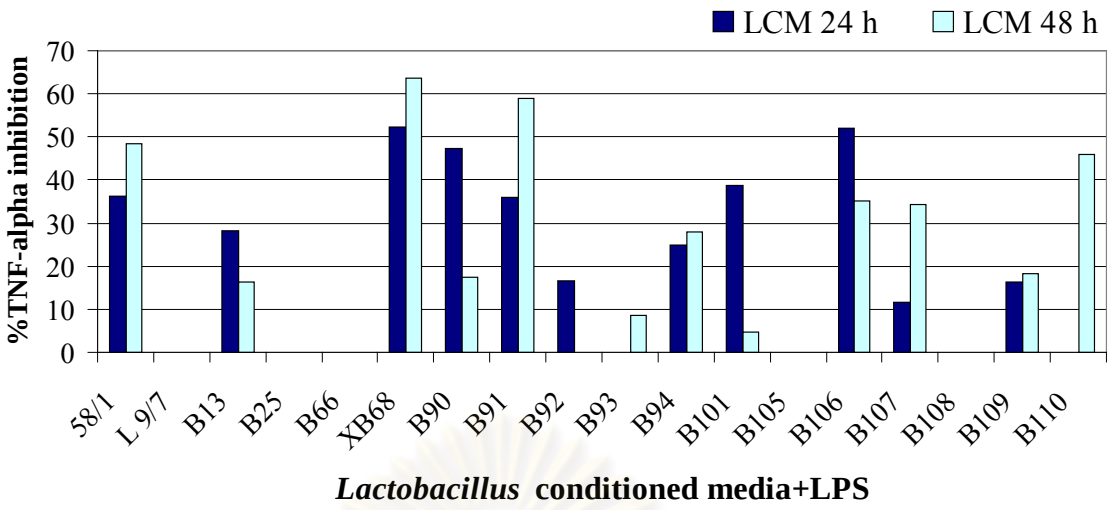


Figure 40. Summary of immunomodulatory effects of 16 *Lactobacillus* isolates from group 1 patients with mild gastritis (9 subjects) on TNF-α production in LPS-stimulated THP-1 monocytic cells. LPS, lipopolysaccharide; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain.

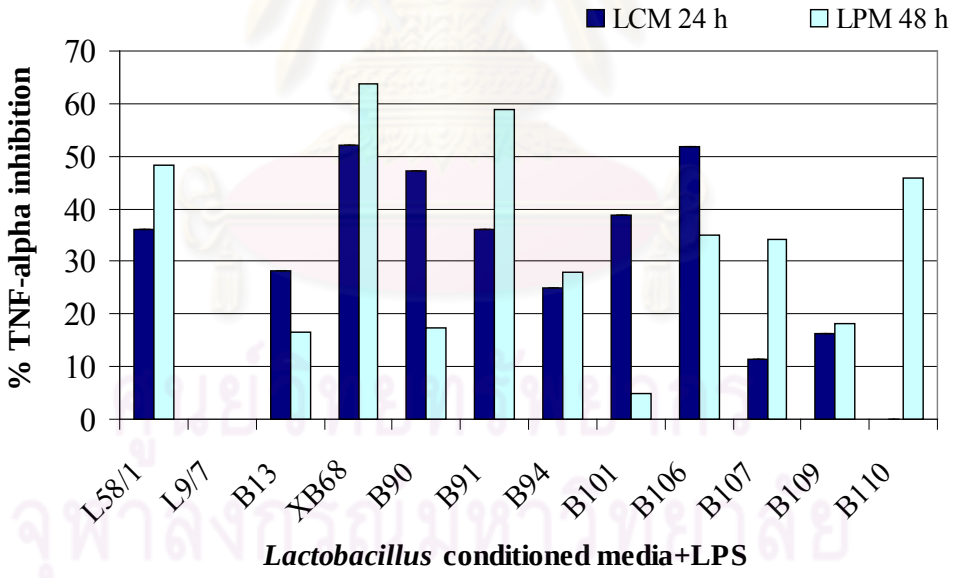


Figure 41. Summary of inhibitory effects of 10 *Lactobacillus* isolates from group 1 patients with mild gastritis (7 subjects) of TNF-α production by LPS-stimulated THP-1 monocytic cells. LPS, lipopolysaccharide; L58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain.

Table 33. Species of *Lactobacillus* isolates with TNF-α inhibitory activity from group 1 patients with mild gastritis.

Colony appearance	**Code**	***Lactobacillus* species**	**Immunomodulatory activity**
M-white (turbid and slime)	B13	*L. casei* group.	TNF-α inhibitory activity
M-white (transparent)	B25	*L. fermentum*	Non-TNF-α inhibitory activity
L-white	B66	*L. fermentum*	Non-TNF-α inhibitory activity
L-white	XB68	*L. gasseri*	TNF-α inhibitory activity
M-yellowish	B90	*L. plantarum*	TNF-α inhibitory activity
L-white turbid	B91	*L. salivarius*	TNF-α inhibitory activity
L-transparent	B92	*L. fermentum*	Non-TNF-α inhibitory activity
M-transparent	XB94	*L. gasseri*	TNF-α inhibitory activity
L-transparent	B93	*L. oris*	Non-TNF-α inhibitory activity
M-whith turbid	B101	*L. salivarius*	TNF-α inhibitory activity
L-transparent	B105	*L. fermentum*	Non-TNF-α inhibitory activity
M-white turbid	B106	*L. casei* group.	TNF-α inhibitory activity
M-white turbid	B107	*L. casei* group.	TNF-α inhibitory activity
M-round transparent	B108	*L. fermentum*	Non-TNF-α inhibitory activity
M-white turbid	B109	*L. salivarius*	TNF-α inhibitory activity
M-turbid	B110	*L. salivarius*	TNF-α inhibitory activity

Table 34. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1435.24±104.88		0.00	1760.95±91.81	
MRS	0.00	1207.38±79.93	-15.88	0.00	1556.43±144.26	-11.61
L58/1	0.00	1053.33±105.41	-12.76	0.00	1233.33±103.00	-20.76
L 9/7	813.81±34.66	1341.43±59.32	11.10	787.38±31.82	1410.71±125.99	-9.36
B2	1308.81±80.8	1457.62±71.94	20.73	1370.71±26.1	1532.14±121.69	-1.56
B6	0.00	898.57±90.95	-25.58	0.00	1002.38±71.79	-35.60
B20	0.00	1267.86±51.49	5.01	0.00	1376.67±229.71	-11.55

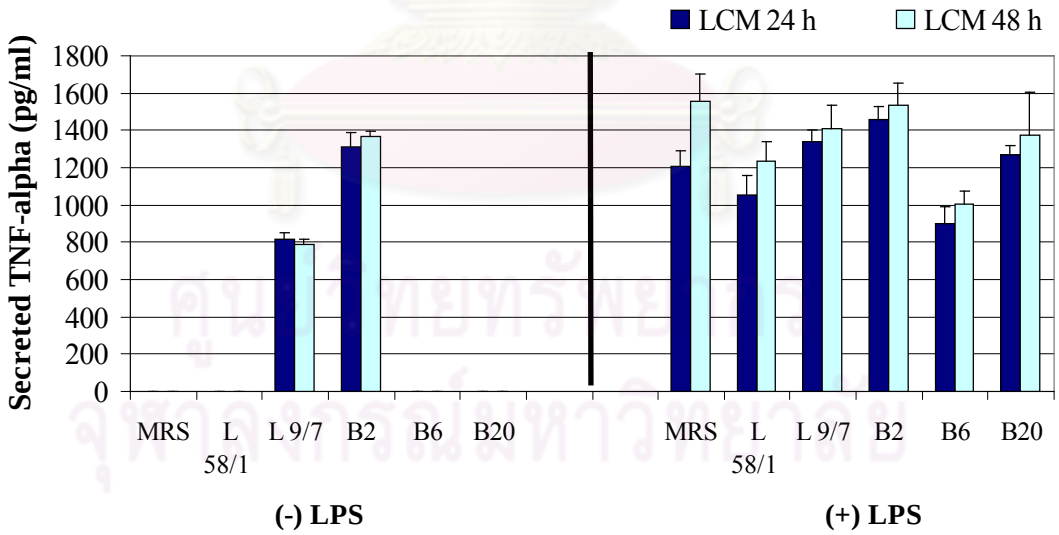


Figure 42. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 35. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1698.13±105.63		0.00	1591.20±173.09	
MRS	0.00	1166.40±60.26	-31.31	0.00	1265.33±89.23	-20.48
L58/1	0.00	800.80±87.30	-31.34	0.00	366.40±278.50	-71.04
L 9/7	1058.13±87.77	1255.73±152.89	7.66	1019.20±25.29	1184.27±163.50	-6.41
B7	0.00	753.33±92.54	-35.41	0.00	322.40±248.35	-74.52
B47	51.47±55.10	1049.33±43.60	-10.04	183.20±180.17	565.07±345.54	-55.34

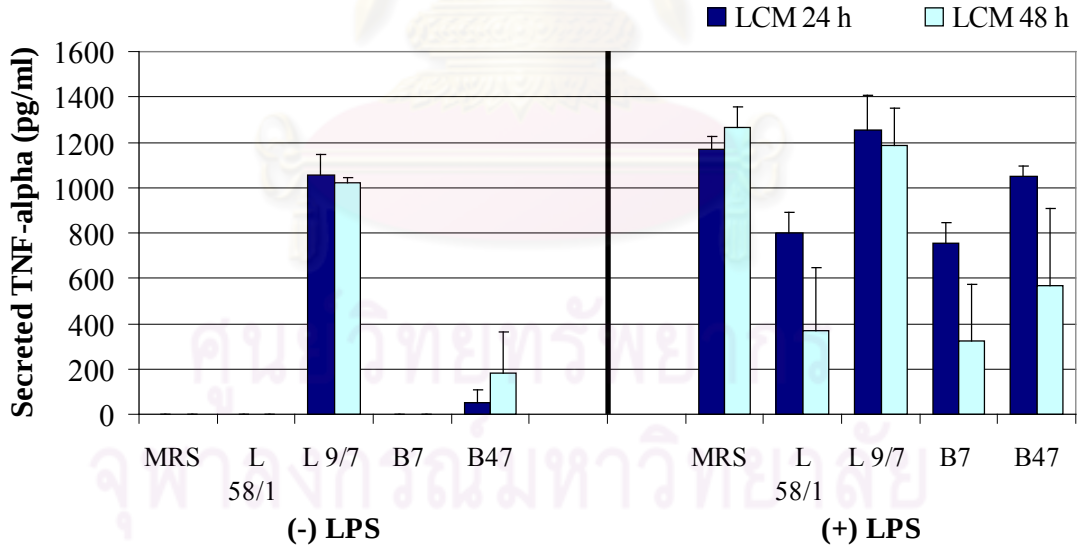


Figure 43. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 36. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1204.57±69.12		0.00	1157.67±54.00	
MRS	0.00	561±75.09	-53.43	0.00	433.38±28.23	-62.56
L58/1	0.00	249.33±70.12	-55.56	0.00	261±76.46	-39.78
L 9/7	737.43±71.92	898.62±39.80	60.18	711.48±34.53	830.29±25.83	91.58
B8	57.90±23.93	386±104.62	-31.19	31.05±53.78	102.67±60.48	-76.31
B70	0.00	293.38±30.74	-47.70	0.00	78.38±97.55	-81.91
XB75	333.62±30.36	652.67±75.19	16.34	305.05±3.52	563.62±102.14	30.05

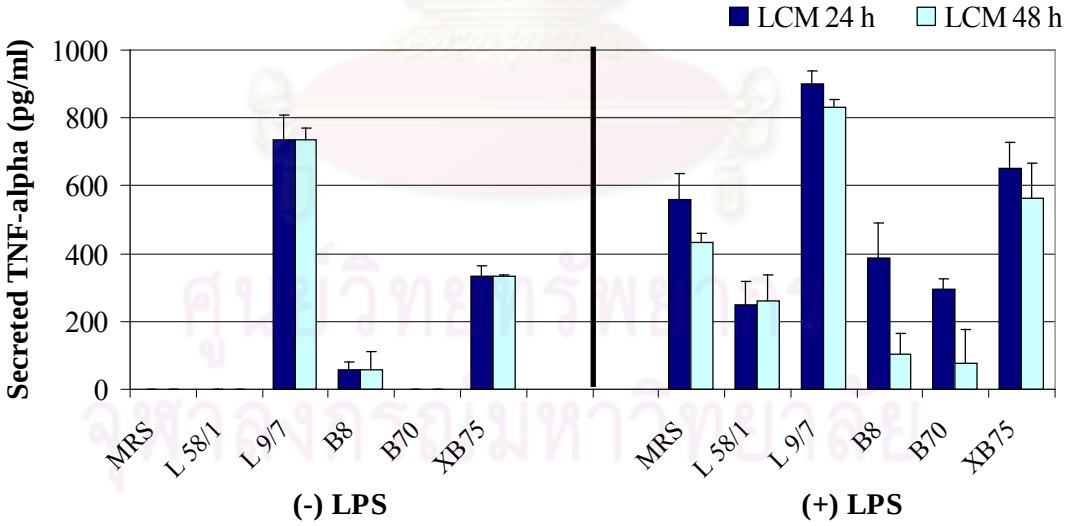


Figure 44. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 37. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	837.52±18.16		0.00	884.19±69.24	
MRS	0.00	628.71±144.29	-24.93	0.00	821.57±69.34	-7.08
L58/1	0.00	476.10±82.63	-24.27	0.00	504.19±153.23	-38.63
L 9/7	620.62±40.00	770.38±37.15	22.53	648.71±63.24	899.67±60.21	9.51
B9	218.24±51.07	769.90±18.37	22.46	252.29±61.88	860.86±92.37	4.78
B39	136.57±26.19	801.81±73.75	27.53	199.67±21.68	793.95±53.74	-3.36
B42	79.67±15.52	690.38±75.40	9.81	200.14±84.65	736.81±56.13	-10.32

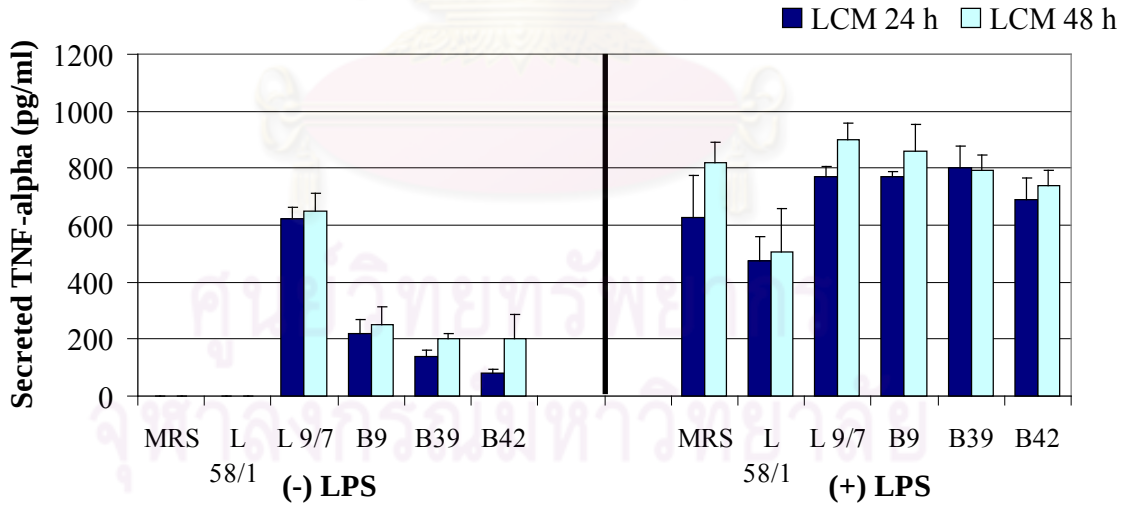


Figure 45. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 38. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	5.58±7.90	1210.46±79.20		16.42±23.53	1091.71±45.02	
MRS	0.00	1037.13±158.23	-14.32	0.00	800.04±18.21	-26.72
L58/1	3.62±6.28	792.13±134.35	-11.57	13.92±21.25	548.38±38.02	-31.46
L 9/7	657.13±7.81	996.71±61.24	-3.90	662.96±33.29	1039.63±102.34	29.95
B18	296.71±97.69	1044.21±146.76	0	484.63±36.31	951.71±216.89	18.96
XB19	334.63±45.43	1061.29±125.03	2.33	165.88±22.53	860.88±63.87	7.60
B21	356.71±32.84	881.71±125.34	-14.99	218.38±3.31	565.46±2.65	-47.47

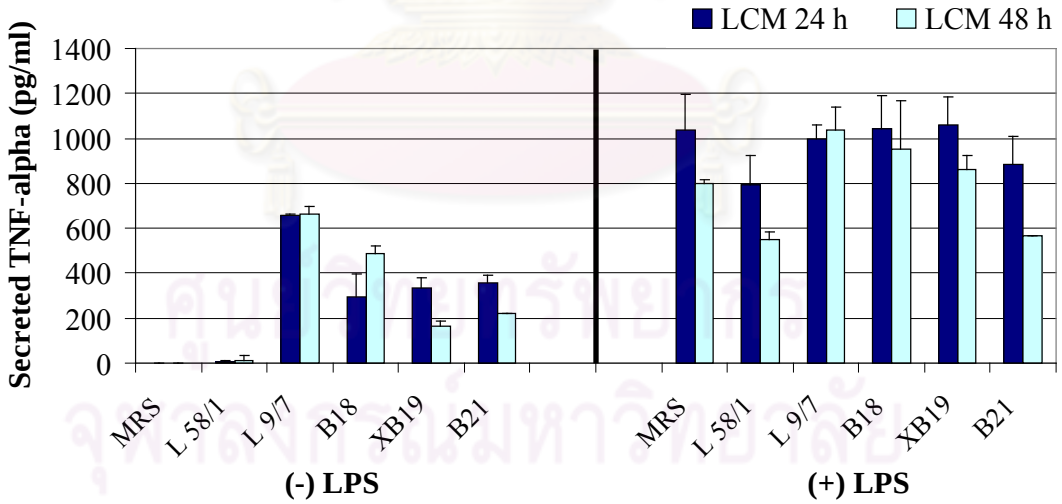


Figure 46. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 39. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1133.89±110.41		0.00	1160.21±71.42	
MRS	0.00	898.11±100.56	-20.79	0.00	759.16±205.28	-34.57
L58/1	0.00	698.81±158.30	-22.19	0.00	589.68±37.94	-22.32
L 9/7	650.74±25.15	1103.72±70.43	22.89	693.19±7.90	944.77±39.18	24.45
B22	764.77±26.47	1233.89±47.59	37.39	825.82±32.64	1011.79±28.30	33.28
B53	111.79±13.93	920.91±111.41	2.54	262.67±14.94	675.3±41.63	-11.05
B54	324.42±64.98	912.84±44.00	1.64	301.96±19.70	784.42±59.93	3.33

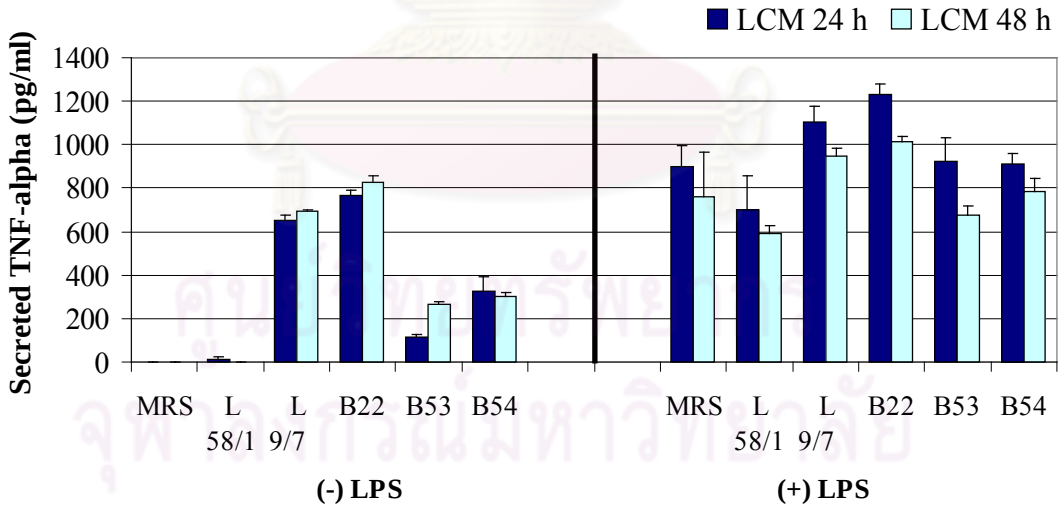


Figure 47. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 40. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1724.29±38.75		0.00	1771.43±65.31	
MRS	0.00	1445.48±96.08	-16.17	0.00	1481.67±64.76	-16.36
L58/1	0.00	1124.29±74.55	-22.22	0.00	1117.86±156.46	-24.55
L 9/7	1324.05±30.48	1507.38±141.95	4.28	1310.00±83.11	1524.52±49.20	2.89
B29	491.67±33.00	1419.52±101.25	-1.80	838.81±6.64	1552.38±25.17	4.77
B31	794.52±20.26	1559.05±59.53	7.86	982.62±36.66	1499.29±93.98	1.19
B35	458.81±57.29	1481.90±175.75	2.52	690.71±12.45	1437.14±37.29	-3.00

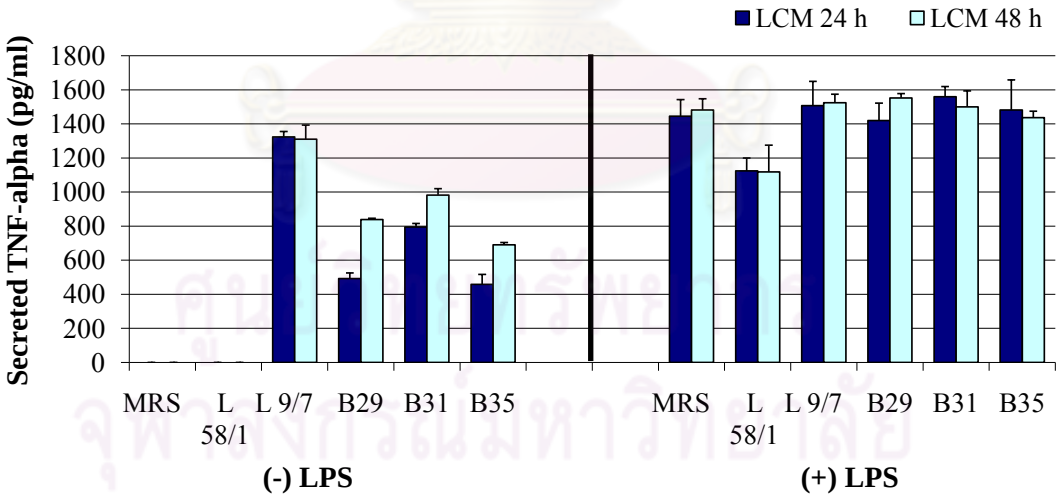


Figure 48. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 41. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	113.6±18.19	1595.60±104.85		77.27±17.39	1685.6±132.28	
MRS	84.93±24.58	1048.27±130.85	-34.30	87.93±17.93	1133.93±48.01	-32.73
L58/1	51.27±9.29	782.27±123.32	-25.38	88.27±34.02	780.93±27.43	-31.13
L 9/7	927.6±54.74	1269.27±16.07	21.08	1021.6±59.81	1300.93±95.92	14.73
B30	587.6±12.29	1102.6±88.54	5.18	410.6±41.76	1167.6±68.35	2.97

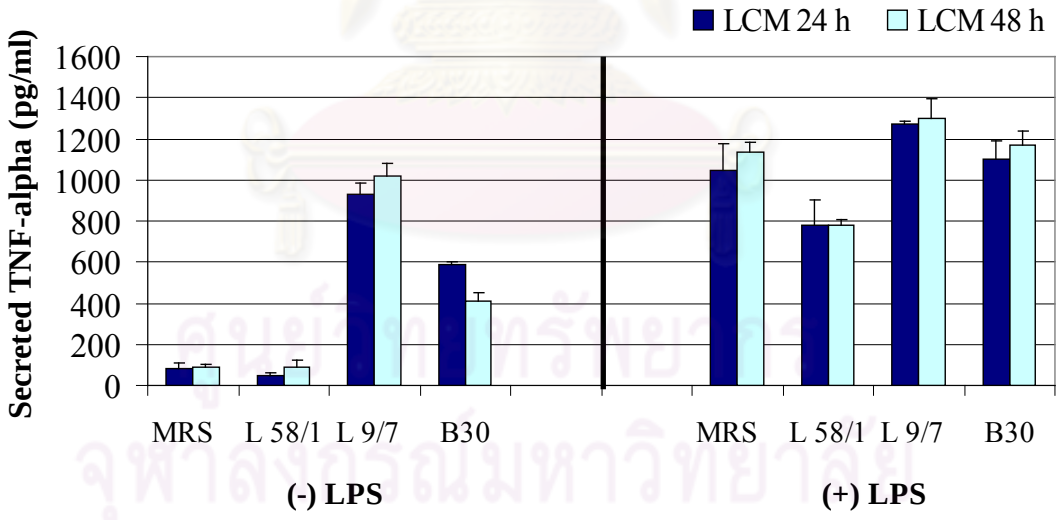


Figure 49. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 42. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1709.16±37.08		0.00	1779.53±59.38	
MRS	0.00	1417.31±84.46	-17.08	0.00	1443.23±106.52	-18.90
L 58/1	0.00	1020.52±143.31	-28.00	0.00	966.44±50.94	-33.04
L 9/7	1314.35±35.50	1589.90±125.06	12.18	1332.37±27.4	1552.37±47.30	7.56
B38	731.63±36.21	1541.26±72.57	8.75	786.94±17.57	1452.37±81.24	0

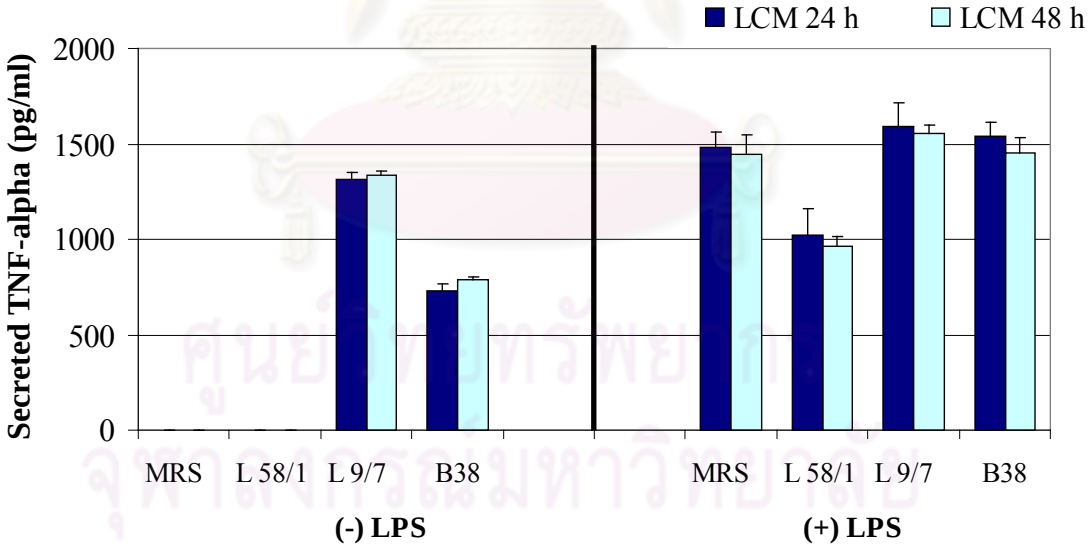


Figure 50. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 43. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1500.07±40.22		0.00	1583.18±57.39	
MRS	0.00	1194.29±96.68	-20.38	0.00	1374.07±155.6	-13.21
L58/1	0.00	793.40±163.27	-33.57	0.00	647.07±30.64	-52.91
L 9/7	1238.07±25.1	1477.18±53.44	23.69	1295.40±15.07	1492.96±68.69	8.65
XB41	0.00	1351.84±46.01	13.19	0.00	959.62±124.16	-30.16
XB49	720.51±48.61	1294.96±123.33	8.43	337.18±35.40	1210.96±190.9	-11.87
B82	832.07±34.43	1460.51±85.14	22.29	948.96±42.90	1458.96±42.49	6.18

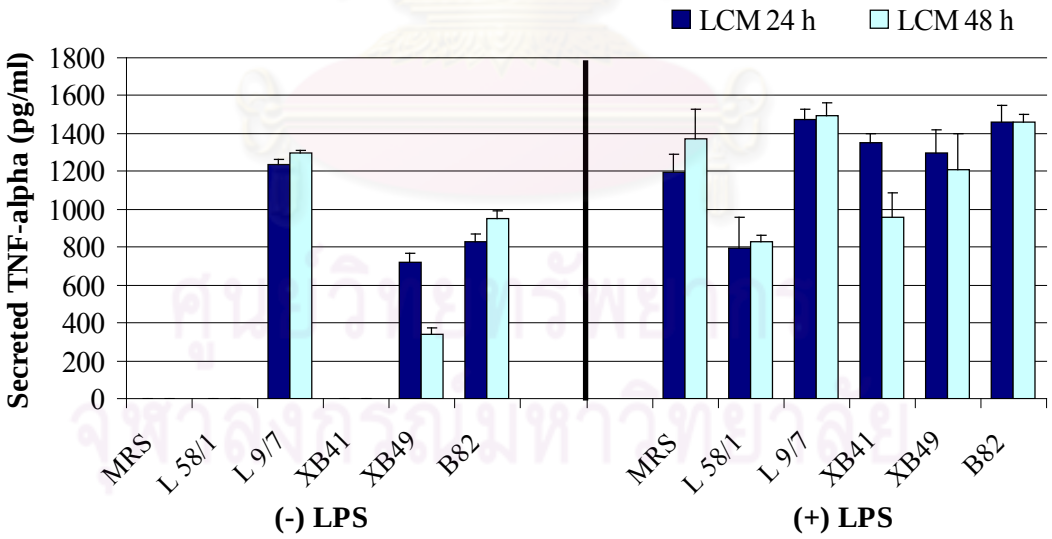


Figure 51. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 44. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1015.64±100.61		0.00	917.45±40.40	
MRS	0.00	787.45±66.48	-22.47	0.00	663.21±28.75	-27.71
L58/1	0.00	372.45±17.36	-52.70	0.00	252.30±61.71	-61.96
L 9/7	570.48±80.19	811.09±87.11	3.00	619.88±62.59	702.61±72.44	5.94
XB45	391.09±29.26	776.55±99.12	-1.39	283.82±31.37	495.33±20.82	-25.31
B48	259.88±29.11	656.55±56.87	-16.62	136.55±26.22	543.52±92.45	-18.05

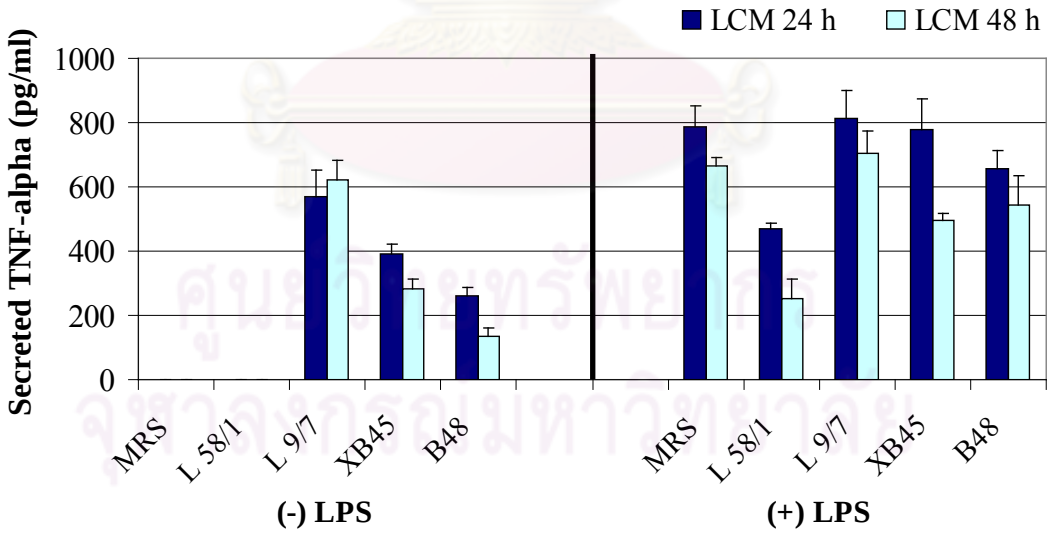


Figure 52. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 45. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1526.87±31.93		0.00	1495.57±48.20	
MRS	0.00	725.13±236.85	-52.51	0.00	685.1±66.13	-54.19
L58/1	0.00	462.81±94.08	-36.18	0.00	531.22±153.96	-22.46
L 9/7	950.93±83.22	1148.61±67.90	58.40	1070.93±71.6	1231.51±73.92	79.76
B46	203.39±41.26	818.75±117.94	12.91	262.52±29.91	938.17±95.86	36.94

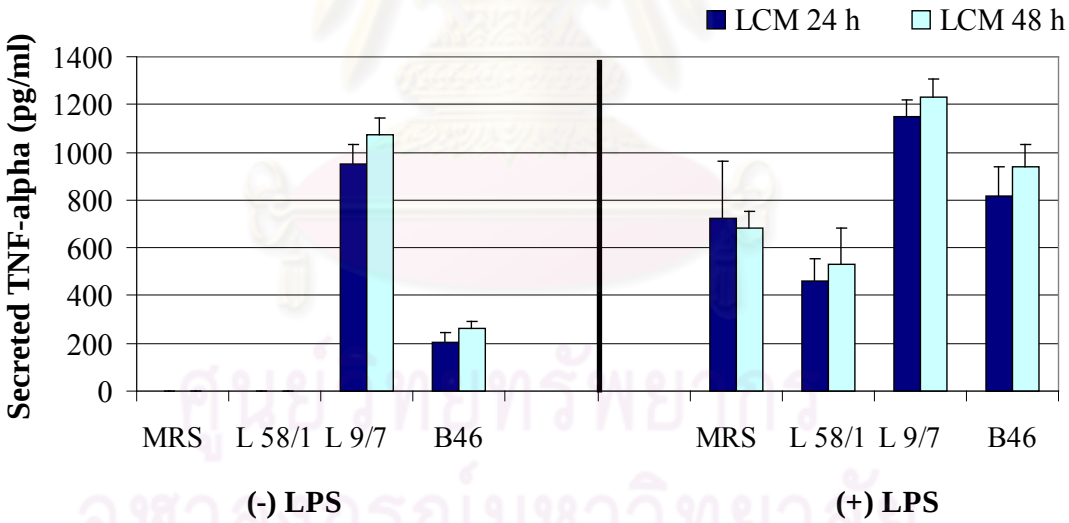


Figure 53. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 46. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1837.59±18.47		0.00	1924.51±172.18	
MRS	0.00	1759.13±43.14	-4.27	0.00	1877.85±84.49	-2.42
L58/1	0.00	1190.41±232.71	-32.33	0.00	1129.13±41.63	-39.87
L 9/7	1404.00±17.74	1724.26±52.36	-1.98	1566.31±55.87	1770.67±31.54	-5.71
B55	85.79±14.48	1584.00±172.26	-9.96	839.38±90.48	1201.95±322.94	-35.99
B76	362.97±33.50	1872.46±42.60	6.44	1130.15±160.20	1700.41±136.17	-9.45

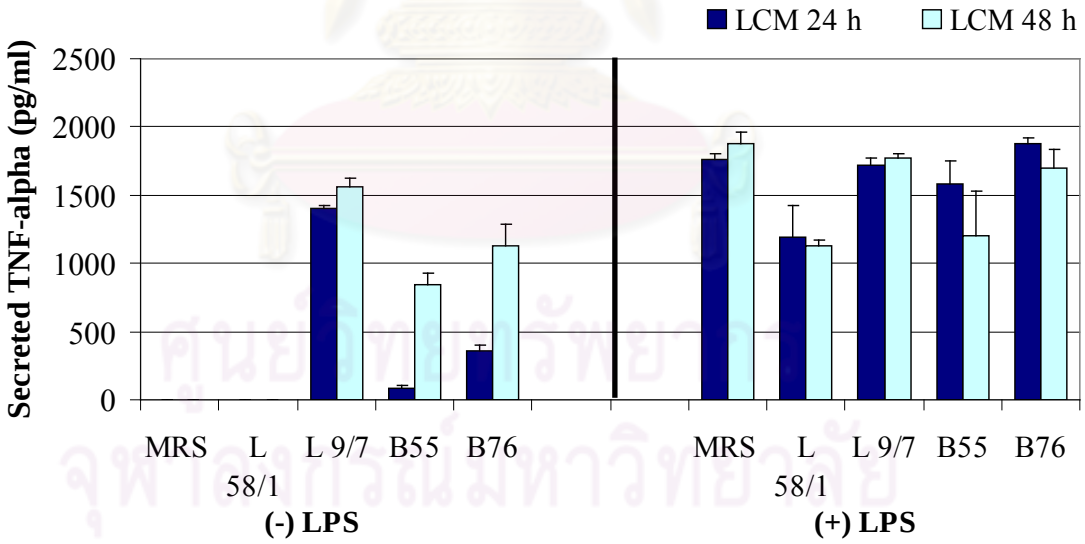


Figure 54. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 47. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1324.76±16.72		0.00	1167.98±181.23	
MRS	0.00	851.89±39.62	-35.70	0.00	691.2±150.35	-40.82
L58/1	0.00	519.47±188.04	-39.02	0.00	390.97±142.25	-43.44
L 9/7	693.03±105	953.49±90.56	11.93	781.08±19.09	938.09±98.14	35.72
XB58	0.00	505.91±237.40	-40.61	0.00	436.48±66.48	-36.85
B60	0.00	814.41±59.68	-4.40	224.53±98.58	359.24±61.67	-48.03
B64	0.00	616.02±64.53	-27.69	0.00	173.49±118.69	-74.90

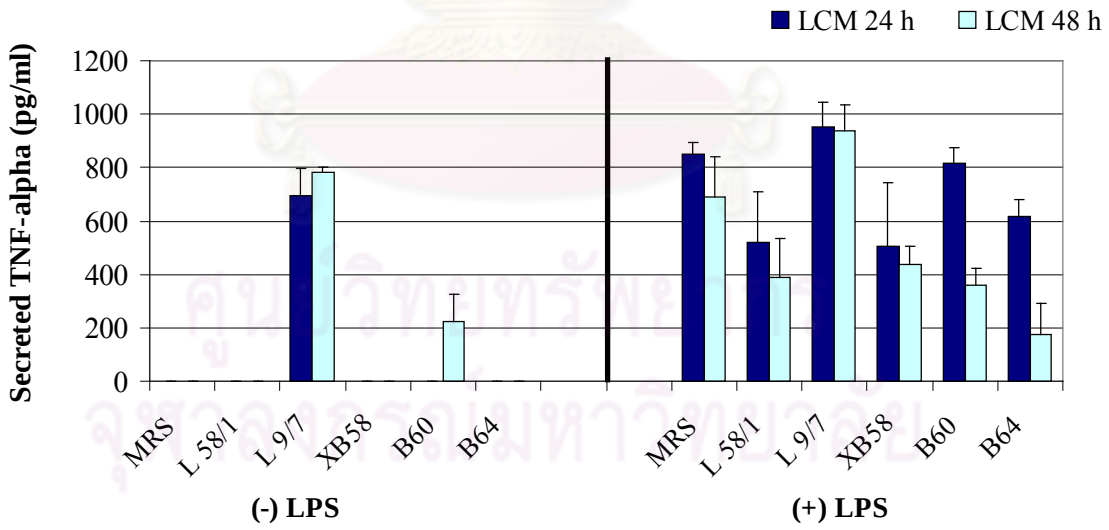


Figure 55. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 48. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1589.75±137.77		0.00	1597.25±167.89	
MRS	0.00	1037.67±20.63	-34.73	0.00	724.33±25.32	-54.65
L58/1	0.00	709.75±179.47	-31.60	0.00	647.25±186.66	-10.64
L 9/7	1082.67±19.1	1271.42±114.30	22.53	1106.42±36.11	1291.83±120.30	78.35
B72	724.75±33.07	1052.25±75.50	1.41	736±8.75	1007.25±40.23	39.06

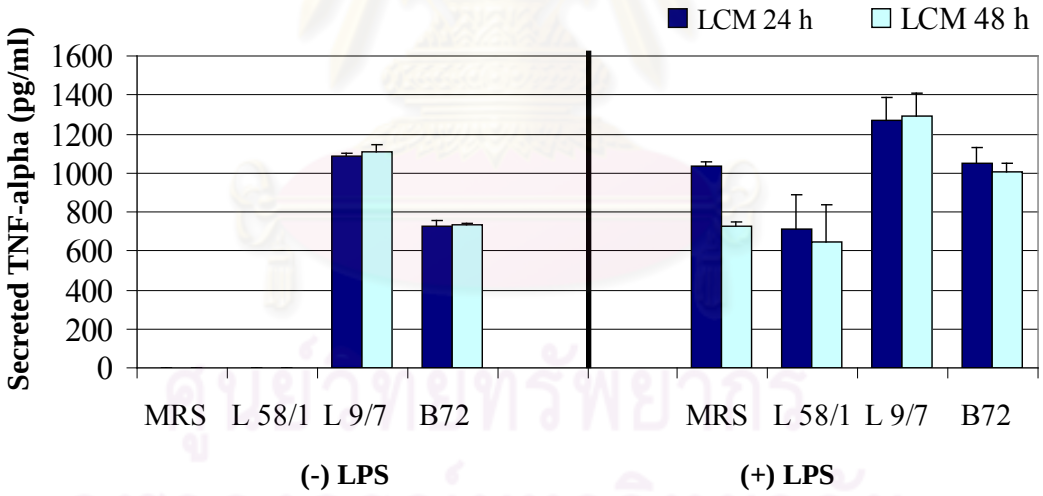


Figure 56. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 49. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1986.71±17.84		0.00	2176.71±397.09	
MRS	0.00	1504.33±76.43	-24.28	0.00	982.9±119.97	-54.84
L 58/1	0.00	1068.62±107.69	-28.96	0.00	489.57±79.50	-50.19
L 9/7	1231.48±140	1562.43±151.97	0.0386	1135.76±112.7	1236.71±200.43	0.2582
B74	0.00	1067.67±61.70	-29.03	139.57±3.78	548.62±77.07	-44.18

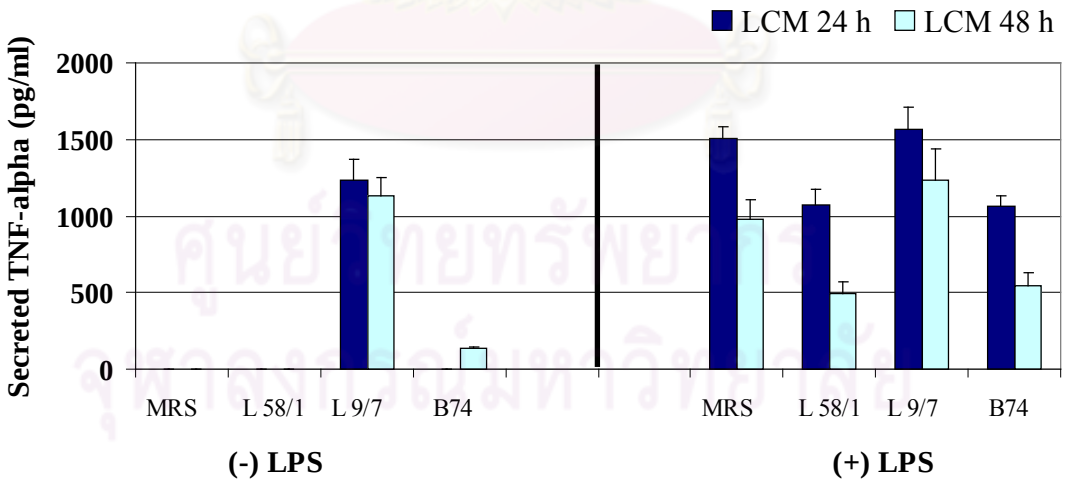


Figure 57. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 50. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1286.43±112.81		0.00	1253.57±21.14	
MRS	0.00	873.81±60.75	-32.07	0.00	821.43±73.11	-34.47
L58/1	0.00	609.29±154.93	-30.27	0.00	418.1±149.05	-49.10
L 9/7	778.57±17.54	1000±130.37	14.44	866.9±8.73	1070.24±32.47	30.29
B67	0.00	654.76±140.86	-25.07	0.00	382.86±157.58	-53.39
B73	242.86±78.24	680.71±86.87	-22.10	151.43±19.18	383.57±109.76	-53.30

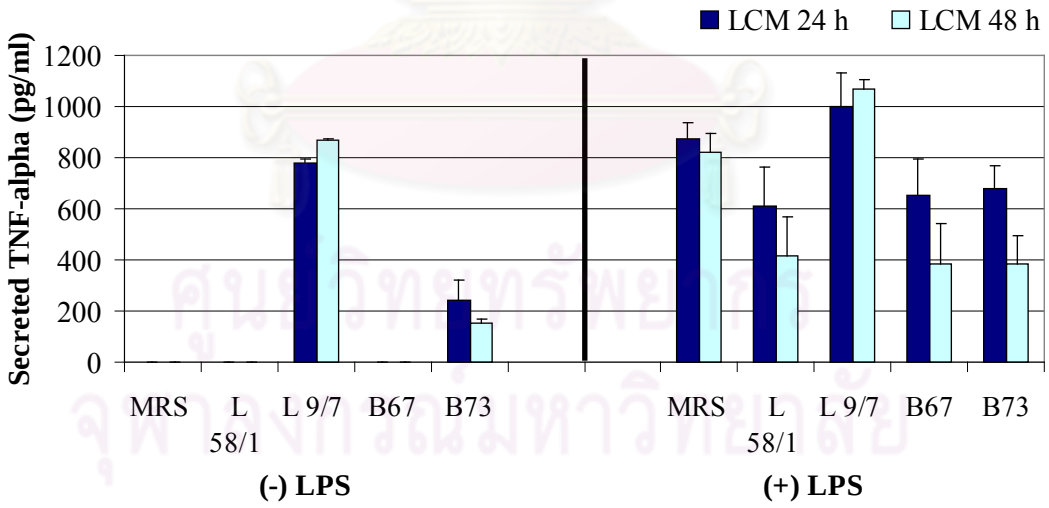


Figure 58. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 51. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1233.67±55.57		0.00	1035.61±179.21	
MRS	0.00	836.72±112.33	-32.18	0.00	586.17±74.03	-43.40
L58/1	0.00	459.50±52.96	-45.08	0.00	221.44±99.59	-62.22
L 9/7	630.61±9.94	914.78±124.69	9.33	680.89±19.53	764.50±79.46	30.42
XB77	70.61±47.29	609.78±40.00	-27.12	80.33±17.46	490.61±41.12	-16.30
B83	387.83±25.51	825.06±70.08	-1.39	321.72±86.35	509.78±91.61	-13.03

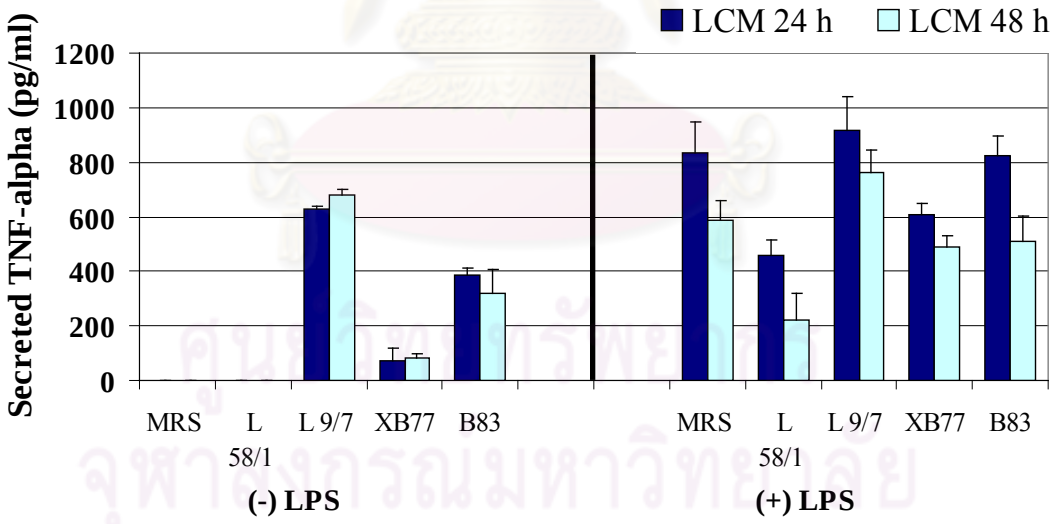


Figure 59. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 52. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1859.98±49.38		0.00	1860.44±21.15	
MRS	0.00	1586.18±195.80	-14.72	0.00	1667.10±51.36	-10.39
L58/1	0.00	1286.18±184.17	-18.91	0.00	779.75±184.43	-53.23
L 9/7	1366.87±112.1	1689.63±70.30	6.52	1366.87±58.0	1697.91±8.23	1.85
B78	181.82±22.96	1530.32±256.43	-3.52	181.82±23.20	1728.02±53.05	3.65

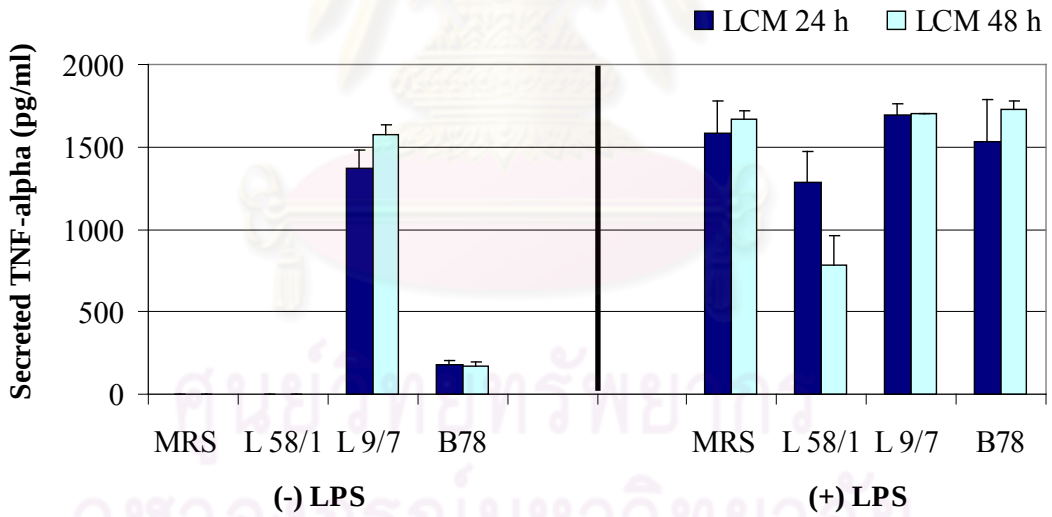


Figure 60. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 53. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1837.59±18.47		0.00	1924.51±172.18	
MRS	0.00	1759.13±43.14	-4.27	0.00	1877.85±84.49	-2.42
L58/1	0.00	1190.41±232.71	-32.33	0.00	1129.13±41.63	-39.87
L 9/7	1404.00±17.74	1724.26±52.36	-1.98	1566.31±55.87	1770.67±31.54	-5.71
B55	85.79±14.48	1584.00±172.26	-9.96	839.38±90.48	1201.95±322.94	-35.99
B59	0.00	1718.62±21.08	-2.30	0.00	1759.64±106.20	-6.29
B76	362.97±33.50	1872.46±42.60	6.44	1130.15±160.20	1700.41±136.17	-9.45

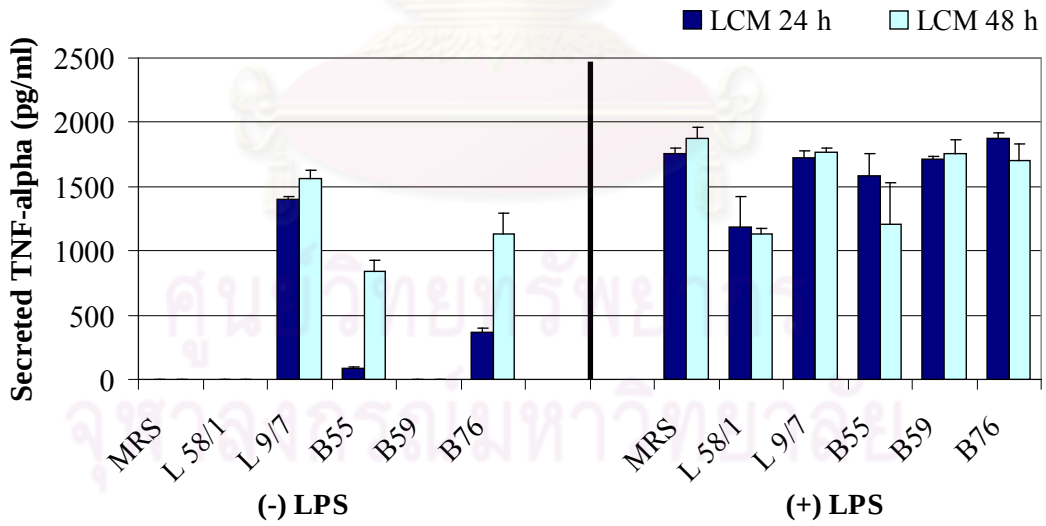


Figure 61. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 54. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	2010.63±125.44		0.00	1729.84±252.46	
MRS	0.00	1324.35±157.71	-34.13	0.00	993.37±246.96	-42.57
L 58/1	0.00	910.63±179.91	-31.24	0.00	565.53±259.28	-45.99
L 9/7	1362.78±50.3	1503.57±143.43	13.53	1154.16±90.2	1284.75±97.19	29.33
B79	568.67±53.87	1595.73±137.32	20.49	705.53±99.60	1176.12±139.59	18.40

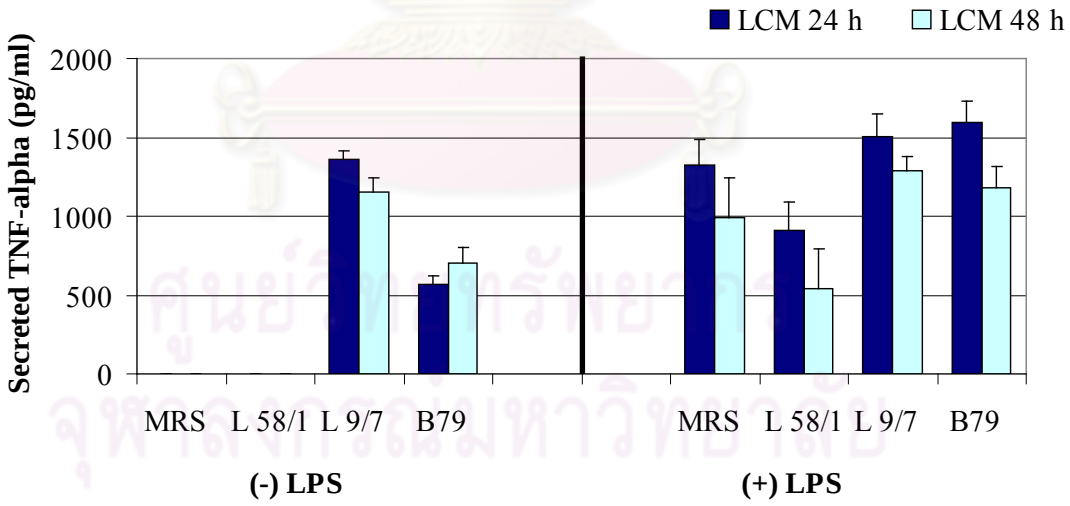


Figure 62. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 55. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1875.73±23.50		0.00	1839.43±97.23	
MRS	0.00	1440.42±68.45	-23.21	0.00	1316.22±45.00	-28.44
L58/1	0.00	1157.21±114.92	-19.66	0.00	538.44±60.63	-59.09
L 9/7	1218.69±61.98	1510.54±46.50	4.87	1293.75±21.11	1366.10±115.02	3.79
B102	467.58±46.40	1342.15±109.20	-6.82	430.79±64.07	804.12±87.95	-38.91
B103	0.00	1445.85±119.33	0.38	0.00	1033.51±27.97	-21.48
B121	679.68±10.79	1467.33±234.20	1.87	1027.58±93.65	1357.21±181.01	3.11

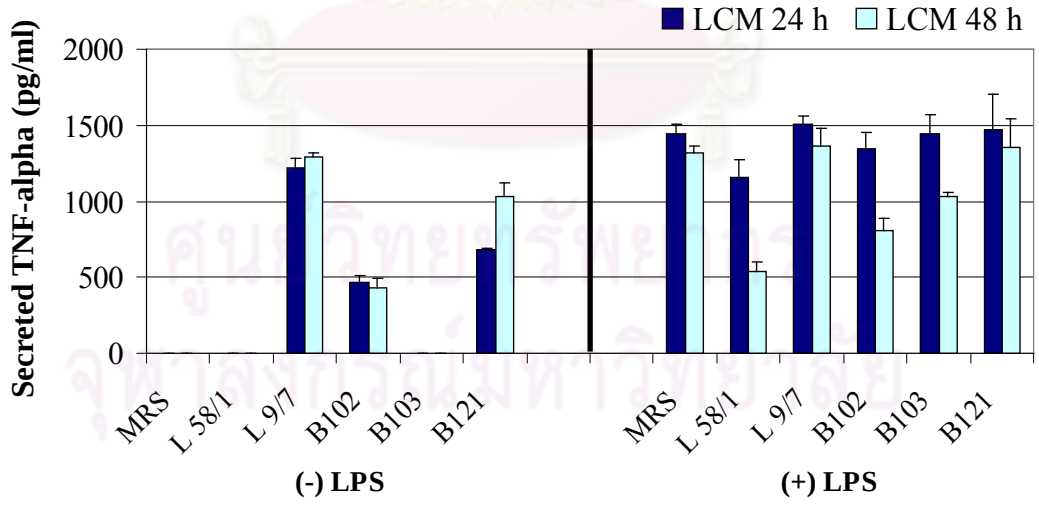


Figure 63. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/ 1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 56. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1590.99±164.13		0.00	1433.20±96.03	
MRS	0.00	1101.45±115.26	-30.77	0.00	1013.23±106.26	-29.30
L 58/1	0.00	569.92±149.01	-48.26	0.00	581.09±24.43	-42.65
L 9/7	1034.32±93.81	1089.43±135.74	-1.09	904.53±177.1	1205.91±65.91	19.02
B87	0.00	342.81±68.64	-68.88	0.00	588.54±96.42	-41.91

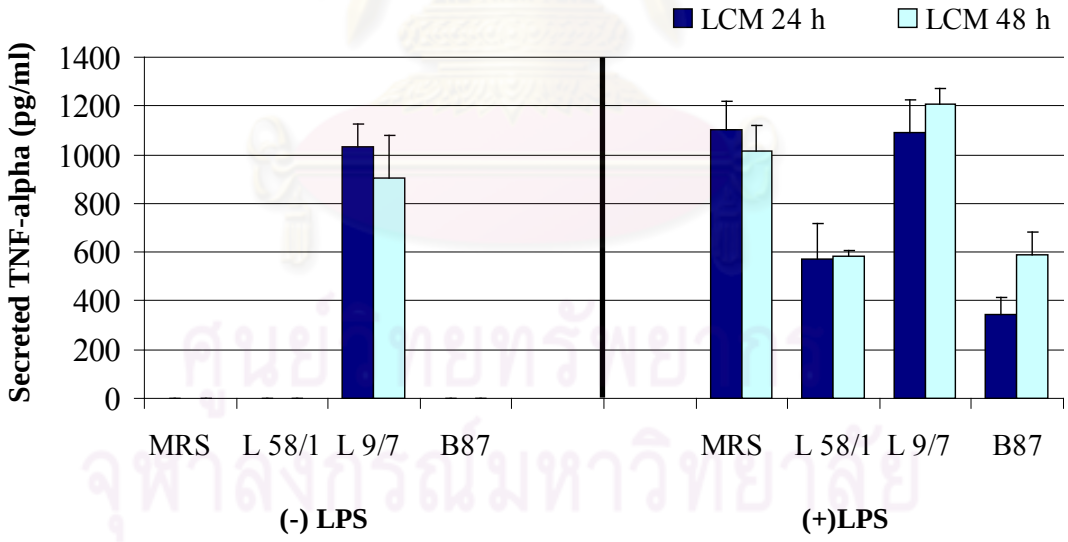


Figure 64. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 57. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1806.16±59.06		0.00	1914.38±31.37	
MRS	0.00	1514.16±89.39	-16.17	0.00	1691.04±48.02	-11.67
L 58/1	0.00	1129.27±98.96	-25.42	0.00	1271.93±146.04	-24.78
L 9/7	1162.82±39.38	1496.38±68.05	-1.17	1183.04±65.6	1584.16±168.05	-6.32
B95	0.00	1358.38±35.08	-10.29	0.00	1272.38±166.13	-24.76
B96	0.00	1387.49±31.37	-8.37	0.00	1112.16±72.73	-34.23

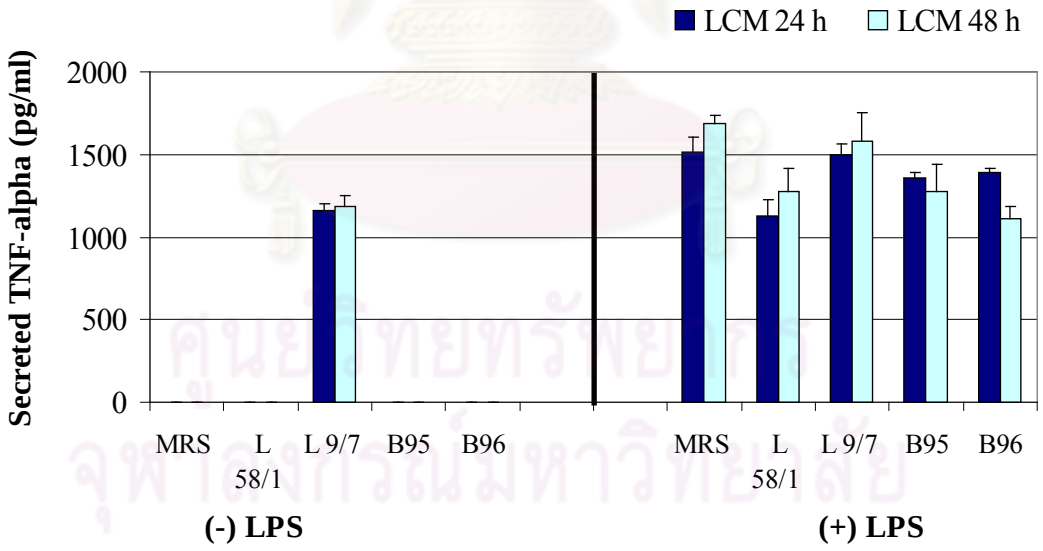


Figure 65. Immunomodulatory effects of *Lactobacillus* isolates from group 2 patients with severe gastritis on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 58. Summary of immunomodulatory effects of 47 *Lactobacillus* isolates from group 2 patients with severe gastritis (32 subjects) on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; % inhibition , - indicated inhibition.

Subject And Patient	**LCM+LPS 24h**	**% TNF-α inhibition**	**P-value**	**LCM+LPS 48h**	**% TNF-α inhibition**	**P-value**
Positive control	L 58/1	-30.86,55.56	<0.05	L 58/1	-40.04,71.04	<0.05
Negative control	L 9/7	14.25	-	L 9/7	20.44	-
1 (26)	B2	20.73	-	B2	-1.56	-
2 (30)	B6	-25.58	<0.01	B6	-35.60	<0.005
3 (43)	B7	-35.41	<0.005	B7	-74.52	<0.005
4 (44)	B8	-31.19	<0.05	B8	-76.31	<0.001
5 (47)	B9	22.46	-	B9	4.78	-
6 (68)	B18 XB19	0 2.33	- -	B18 XB19	18.96 7.60	- -
7 (70)	B20	5.01	-	B20	-11.55	-
8 (73)	B21 B22	-14.98 37.39	- -	B21 B22	-47.47 33.28	<0.001 -
9 (94)	B29	-1.80	-	B29	4.77	-
10 (95)	B31	7.86	-	B31	1.19	-
11 (96)	XB30 B35	5.18 2.52	- -	XB30 B35	2.97 -3	- -
12 (110)	B38 B39	8.75 27.53	- -	B38 B39	0 -3.36	- -
13 (105)	XB41	13.19	-	XB41	-30.16	<0.05
14 (120)	B42	9.81	-	B42	-10.32	-
15 (121)	XB45	-1.39	-	XB45	-25.31	<0.05

Table 58. Summary of immunomodulatory effects of 47 *Lactobacillus* isolates from group 2 patients with severe gastritis (32 subjects) on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; % inhibition , - indicated inhibition. (Continued)

Subject And Patient	LCM+LPS 24h	% TNF-α inhibition	P-value	LCM+LPS 48h	% TNF-α inhibition	P-value
16 (132)	B46	12.91	-	B46	36.94	-
	B47	-10.04	<0.05	B47	-55.34	<0.05
17 (135)	XB48	-16.62	<0.05	XB48	-18.05	<0.05
18 (137)	XB49	8.43	-	XB49	-11.87	-
19 (153)	B53	2.54	-	B53	-11.05	-
	B54	1.64	-	B54	3.33	-
20 (155)	B55	-9.96	-	B55	-35.99	<0.05
21 (154)	XB58	-40.61	<0.05	XB58	-36.85	<0.05
	B60	-4.40	-	B60	-48.03	<0.05
	B64	-27.69	<0.005	B64	-74.90	<0.005
22 (165)	B67	-30.08	<0.05	B67	-39.86	<0.05
23 (175)	B70	-47.70	<0.005	B70	-81.91	<0.005
24 (185)	B72	1.41	-	B72	39.46	-
	B73	-22.10	<0.05	B73	-53.30	<0.005
25 (190)	B74	-29.03	<0.001	B74	-44.18	<0.05
	XB75	16.34	-	XB75	30.05	-
	XB77	-27.12	<0.05	XB77	-16.30	-
	B78	-3.52	-	B78	3.56	-
26 (187)	B76	6.44	-	B76	-9.45	-
27 (192)	B79	20.49	-	B79	18.40	-
	B121	1.87	-	B121	3.11	-

Table 58. Summary of immunomodulatory effects of 47 *Lactobacillus* isolates from group 2 patients with severe gastritis (32 subjects) on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; % inhibition, - indicated inhibition. (Continued)

Subject And Patient	LCM+LPS 24h	% TNF-α inhibition	P-value	LCM+LPS 48h	% TNF-α inhibition	P-value
28 (200)	B82	22.29	-	B82	6.18	-
	B83	-1.39	-	B83	-13.03	-
29 (210)	B87	-68.88	<0.0005	B87	-41.91	<0.005
30 (235)	XB95	-10.29	<0.05	XB95	-24.76	<0.01
31 (232)	XB96	-8.37	<0.005	XB96	-34.23	<0.0005
32 (276)	B102	-6.82	-	B102	-38.91	<0.0005
	B103	0.38	-	B103	-21.48	<0.0005

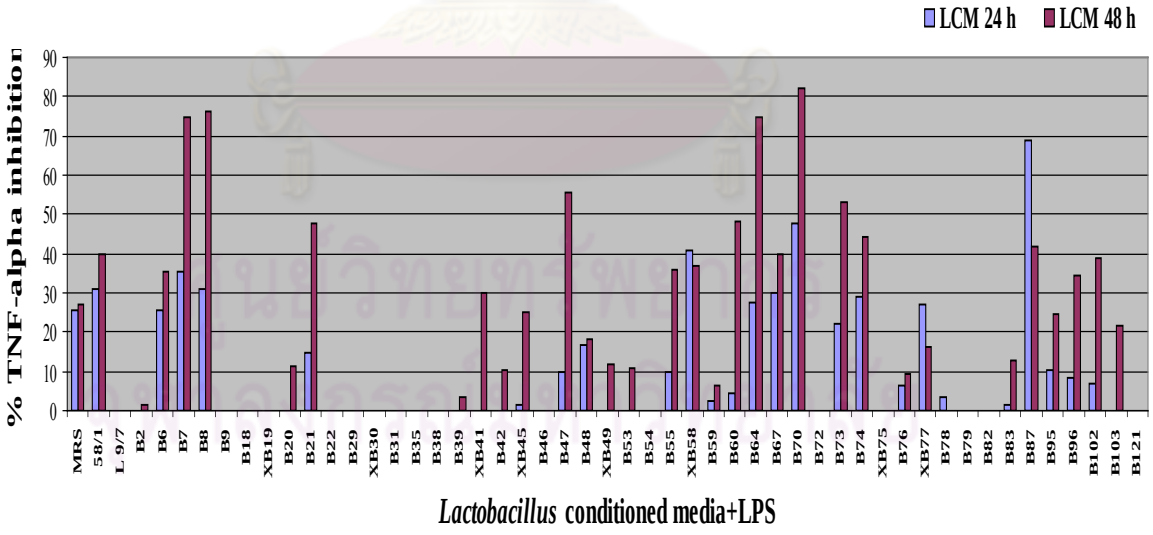


Figure 66. Summary of inhibitory effects of 47 *Lactobacillus* isolates from group 2 patients with severe gastritis (32 subjects) of TNF-α production by LPS-stimulated THP-1 monocytic cells. LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain.

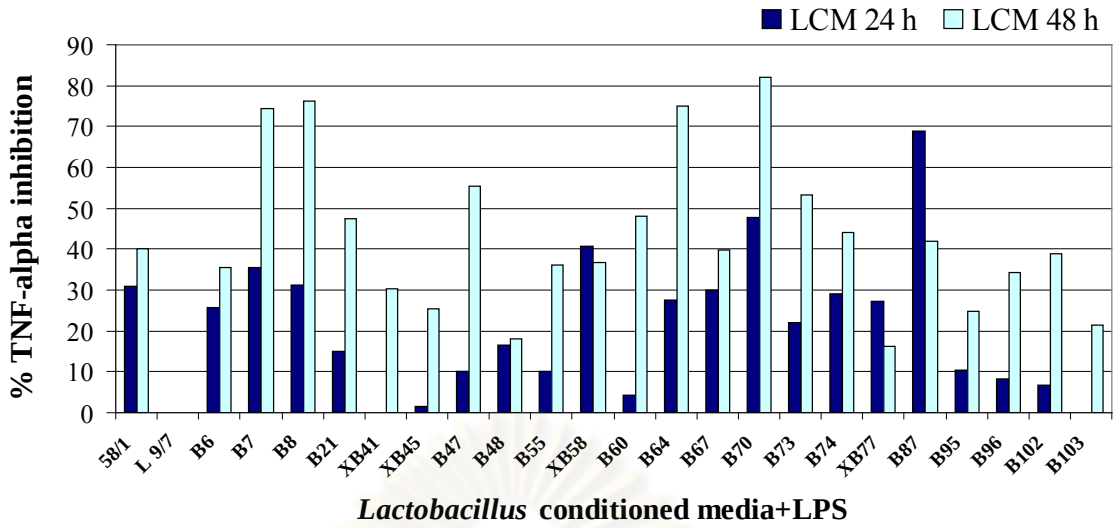


Figure 67. Summary of inhibitory effects of 22 *Lactobacillus* isolates from group 2 patients with severe gastritis (18 subjects) of TNF-α production by LPS-stimulated THP-1 monocytic cells. LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain.

Table 59. Species of *Lactobacillus* isolates with TNF-α inhibitory activity from group 2 patients with severe gastritis.

Colony appearance	Code	*Lactobacillus* species	Immunomodulatory activity
L-white turbid	B2	*L. fermentum*	Non-TNF-α inhibitory activity
L-white turbid	B6	*L. plantarum*	TNF-α inhibitory activity
L-white turbid	B7	*L. plantarum*	TNF-α inhibitory activity
L-white turbid	B8	*L. salivarius*	TNF-α inhibitory activity
S-transparent	B9	*L. fermentum*	Non-TNF-α inhibitory activity
L-white transparent	B18	*L. oris*	Non-TNF-α inhibitory activity
M-white	XB19	*L. gasseri*	Non-TNF-α inhibitory activity
L-white transparent	B20	*L. fermentum*	Non-TNF-α inhibitory activity
M-white turbid	B21	*L. salivarius*	TNF-α inhibitory activity
M-white transparent	B22	*L. oris*	Non-TNF-α inhibitory activity
L-white ใส	B29	*L. fermentum*	Non-TNF-α inhibitory activity
M-white	B31	*L. fermentum*	Non-TNF-α inhibitory activity
S-white	XB30	*L. gasseri*	Non-TNF-α inhibitory activity
M-white transparent	B35	*L. fermentum*	Non-TNF-α inhibitory activity
L-white transparent	B38	*L. fermentum*	Non-TNF-α inhibitory activity
M-transparent	B39	*L. fermentum*	Non-TNF-α inhibitory activity
S-turbid	XB41	*L. gasseri*	TNF-α inhibitory activity
M-white transparent	B42	*L. fermentum*	Non-TNF-α inhibitory activity
M-transparent	XB45	*L. gasseri*	TNF-α inhibitory activity
M-white transparent	B46	*L. fermentum*	Non-TNF-α inhibitory activity
M-white transparent	B47	*L. salivarius*	TNF-α inhibitory activity
M-white	XB48	*L. gasseri*	TNF-α inhibitory activity
M-transparent	XB49	*L. gasseri*	Non-TNF-α inhibitory activity
M-round turbid	B53	*L. salivarius*	Non-TNF-α inhibitory activity
M-round transparent	B54	*L. mucosae*	Non-TNF-α inhibitory activity
M-turbid	B55	*L. salivarius*	TNF-α inhibitory activity

Table 59. Species of *Lactobacillus* isolates with TNF-α inhibitory activity from group 2 patients with severe gastritis. (Continued)

Colony appearance	Code	*Lactobacillus* species	Immunomodulatory activity
M-transparent	XB58	*L. gasseri*	TNF-α inhibitory activity
M-yellowish	B64	*L. plantarum*	TNF-α inhibitory activity
M-white	B60	*L. salivarius*	TNF-α inhibitory activity
L-yellowish	B67	*L. plantarum*	TNF-α inhibitory activity
M-yellowish	B70	*L. plantarum*	TNF-α inhibitory activity
M- turbid	B72	*L. fermentum*	Non-TNF-α inhibitory activity
M- turbid	B73	*L. salivarius*	TNF-α inhibitory activity
L- turbid	B74	*L. salivarius*	TNF-α inhibitory activity
M-round transparent	XB75	*L. fermentum*	Non-TNF-α inhibitory activity
M-transparent	XB77	*L. gasseri*	TNF-α inhibitory activity
M-white turbid	B78	*L. salivarius*	Non-TNF-α inhibitory activity
L-white transparent	76	*L. fermentum*	Non-TNF-α inhibitory activity
L-transparent	B79	*L. mucosae*	Non-TNF-α inhibitory activity
M-turbid	B121	*L. mucosae*	Non-TNF-α inhibitory activity
L-transparent	B82	*L. fermentum*	Non-TNF-α inhibitory activity
M-transparent	B83	*L. fermentum*	Non-TNF-α inhibitory activity
M-yellowish	B87	*L. plantarum*	TNF-α inhibitory activity
M-white	XB95	*L. gasseri*	TNF-α inhibitory activity
M-white	XB96	*L. gasseri*	TNF-α inhibitory activity
M-white turbid	B102	*L. salivarius*	TNF-α inhibitory activity
M-white	B103	*L. casei*	TNF-α inhibitory activity

Table 60. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1525.96±66.32		0.00	1485.07±140.97	
MRS	0.00	1121.96±115.93	-26.48	0.00	851.73±177.45	-42.65
L 58/1	0.00	724.18±156.07	-35.45	0.00	400.4±134.82	-52.99
L 9/7	1083.73±132.50	1330.84±58.64	18.62	1037.51±132.9	1281.96±58.67	50.51
B4/2	462.4±58.48	1051.73±185.51	-6.26	467.73±67.03	890.84±45.59	4.59
B5	442.84±5.05	1144.62±155.82	2.02	341.51±66.68	1000.62±147.43	17.48

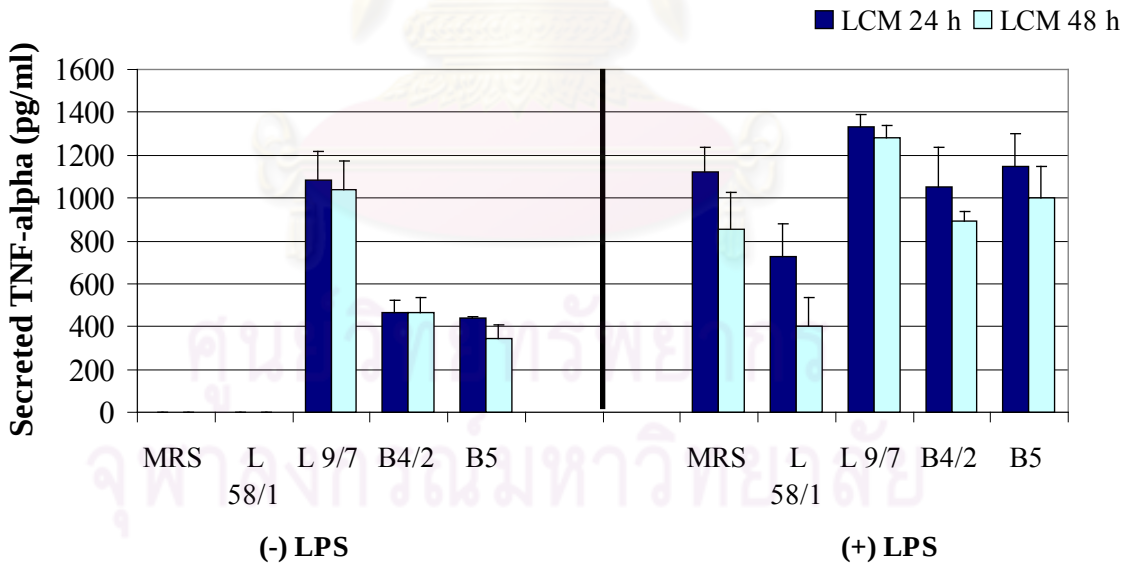


Figure 68. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 61. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1837.14±37.80		0.00	1459.52±46.95	
MRS	0.00	1104.29±228.42	-39.89	0.00	838.1±106.58	-42.58
L58/1	21.9±23.75	630.95±102.56	-42.86	0.00	599.05±66.04	-28.52
L 9/7	1245.71±90.7	1530.95±103.10	38.64	1144.29±67.6	1309.52±139.2	56.25
B15	925.24±58.94	1571.9±64.08	42.35	876.19±53.57	1097.14±21.33	30.91

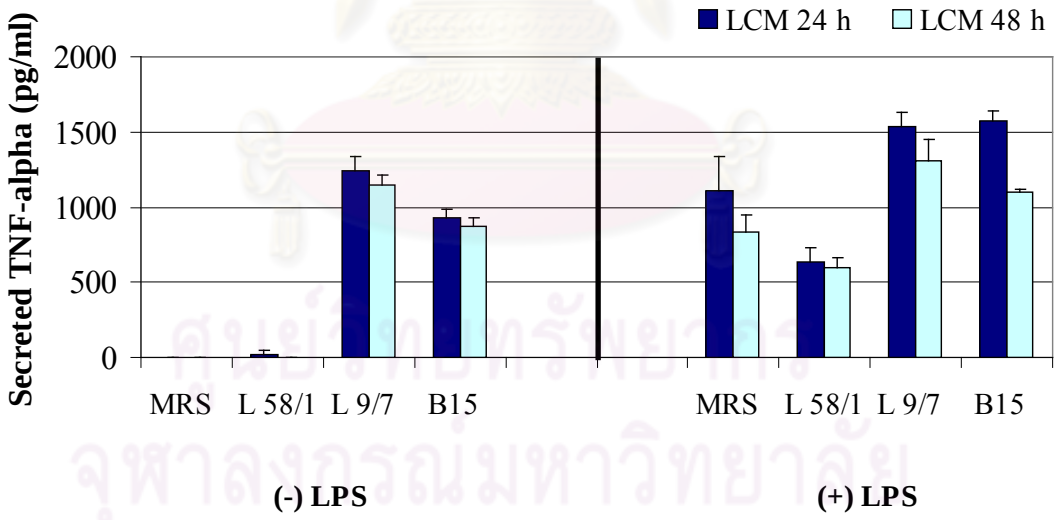


Figure 69. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 62. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1272.71±158.69		0.00	1326.05±33.83	
MRS	0.00	541.22±48.20	-57.48	0.00	563.98±36.83	-57.47
L58/1	0.00	153.86±37.11	-71.57	0.00	116.62±45.39	-79.32
L 9/7	615.24±46.71	845.36±83.54	56.20	735.47±174.81	999.84±111.89	77.28
XB7	0.00	123.06±40.43	-77.26	0.00	241.22±66.09	-57.23
B23	35.93±7.95	475.93±86.22	-12.06	0.00	340.53±87.06	-39.62

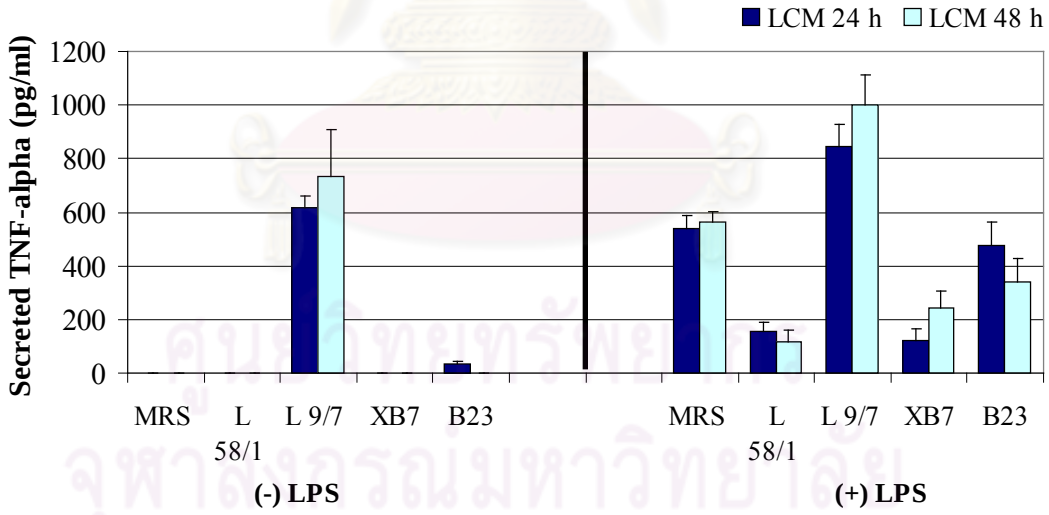


Figure 70. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 63. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1585.31±42.65		0.00	1488.75±30.27	
MRS	0.00	1273.70±75.11	-19.66	0.00	1203.38±40.12	-19.17
L58/1	0.00	840.15±105.57	-34.04	0.00	738.97±40.60	-38.59
L 9/7	1230.69±23.17	1416.06±8.46	11.18	1230.47±25.62	1401.01±70.82	16.42
B14	851.12±6.53	1371.76±98.54	7.70	988.11±27.81	1343.59±67.69	11.65
B24	447.03±79.43	1376.92±56.97	8.10	373.05±113.61	1380.80±40.07	14.74
B98	776.28±51.23	1509.61±65.44	18.52	717.78±58.05	1301.44±54.52	8.15

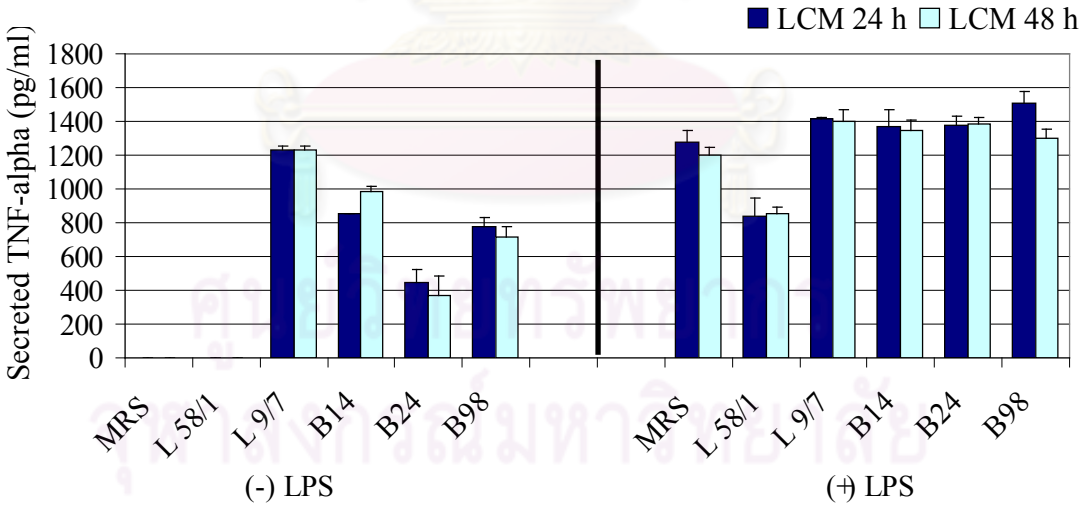


Figure 71. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 64. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1949.96±57.96		0.00	2123.29±226.99	
MRS	0.00	1569.07±83.98	-19.53	0.00	1247.73±275.16	-41.24
L 58/1	0.00	931.73±321.76	-40.62	0.00	948.18±206.49	-24.01
L 9/7	1422.84±49.64	1545.07±146.68	0	1306.84±78.50	1596.18±102.00	27.93
B16	307.29±113.03	1470.4±187.71	-6.29	626.89±71.38	1477.07±343.61	18.38
B26	1001.96±190.81	1527.29±104.31	-2.66	1194.4±61.33	1439.73±146.83	15.39

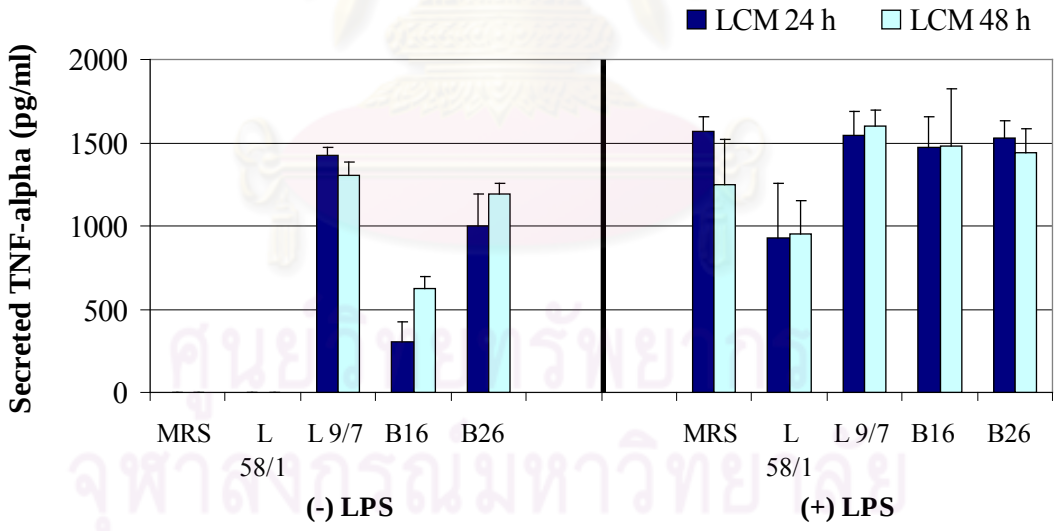


Figure 72. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 65. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	813.69±33.61		0.00	967.02±141.40	
MRS	0.00	654.17±161.44	-19.60	0.00	900.6±181.82	-6.87
L58/1	0.00	529.64±30.36	-19.04	0.00	449.17±184.18	-50.13
L 9/7	573.21±41.79	682.5±85.22	4.33	607.26±41.79	838.69±225.15	-6.87
B32	58.45±34.75	580.36±62.34	-11.28	452.98±34.75	861.79±91.12	-4.31
B37	151.07±39.46	636.55±65.67	-2.69	257.74±39.46	621.07±170.68	-31.04
B61	157.5±8.95	821.79±73.90	25.62	148.93±8.95	884.88±156.51	-1.74

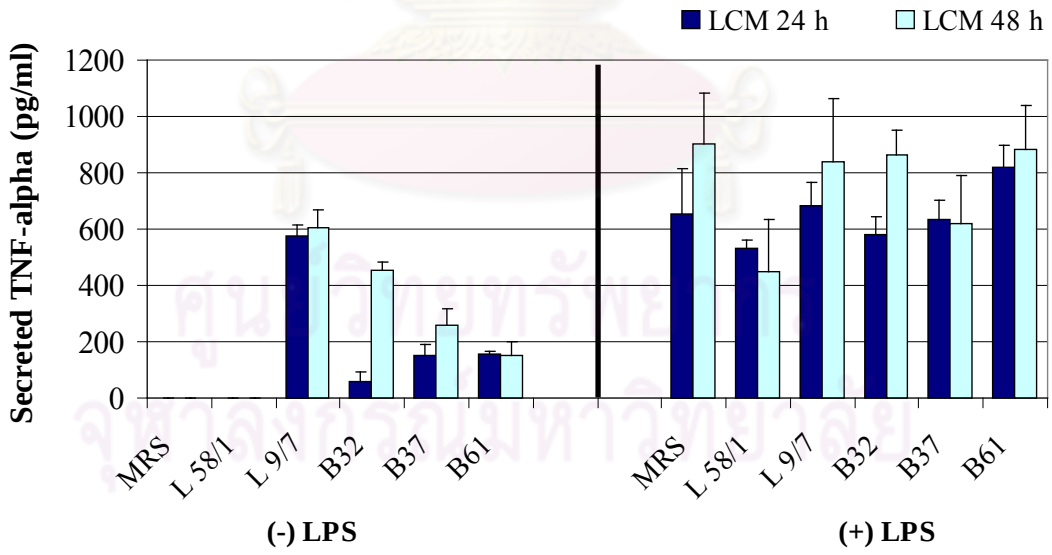


Figure 73. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 66. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1559.58±98.38		0.00	1595.42±155.16	
MRS	0.00	1065.83±56.71	-31.66	0.00	1068.33±106.07	-33.04
L58/1	0.00	807.08±144.47	-24.28	0.00	563.75±241.41	-47.23
L 9/7	994.17±10.03	1333.75±106.98	25.14	1041.25±26.52	1208.75±88.78	13.14
B33	627.08±43.16	1074.58±39.02	0.82	818.33±71.42	1179.58±325.71	10.41
B43	505.42±77.06	1057.08±154.46	0	585.83±34.13	872.5±146.43	-18.33
B44	703.75±46.59	1225.92±140.77	15.02	627.92±42.30	1220±146.79	14.20

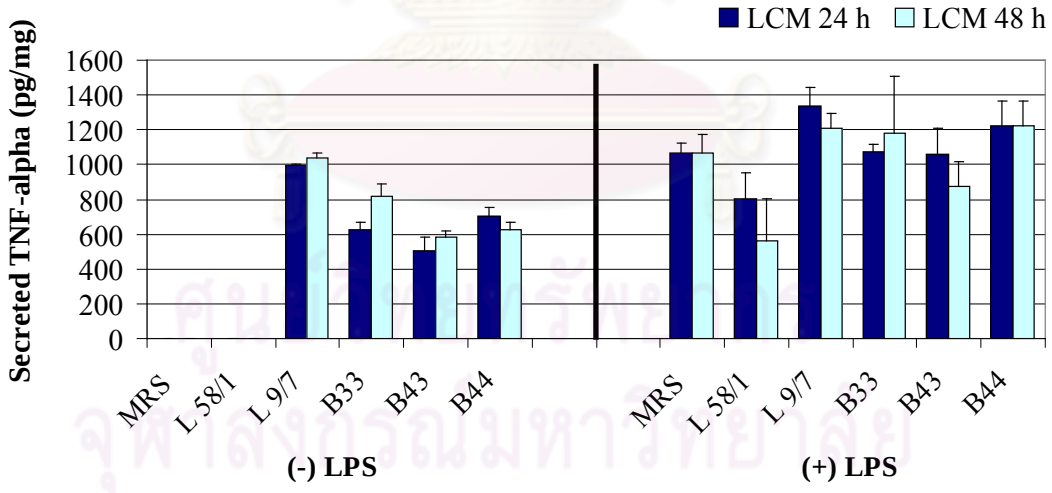


Figure 74. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 67. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1458.19±114.88		0.00	1324.05±114.14	
MRS	0.00	788.59±162.40	-45.92	0.00	630.99±105.36	-52.34
L58/1	0.00	376.85±45.05	-52.21	0.00	133.39±33.08	-78.86
L 9/7	764.85±78.67	925.39±104.63	17.35	774.99±27.32	884.32±67.15	40.15
B36	0.00	293.65±119.97	-62.76	0.00	529.39±153.94	-16.10
B37	325.92±18.62	648.32±125.73	-17.79	303.25±86.01	467.79±95.59	-25.86
XB40	0.00	368.85±180.25	-53.23	0.00	110.99±35.10	-82.41

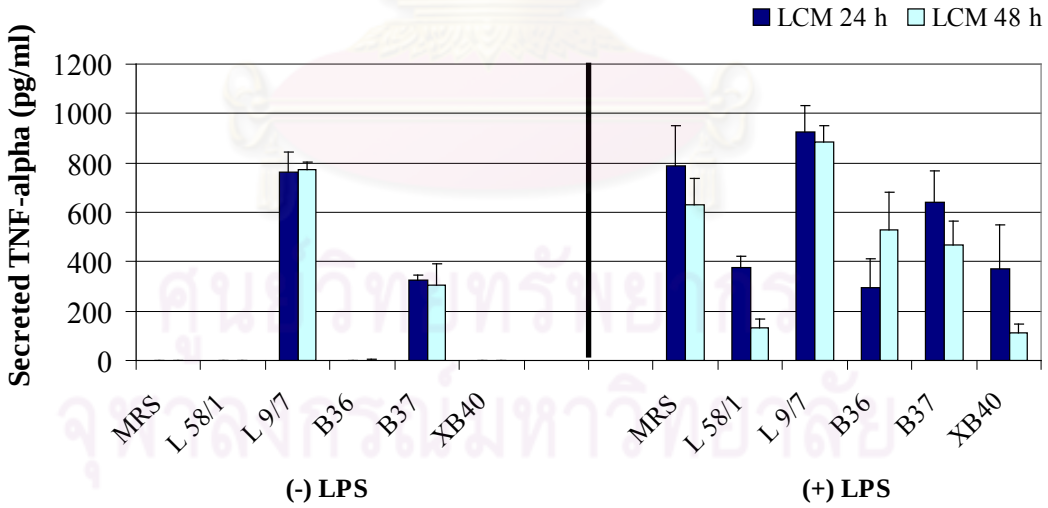


Figure 75. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 68. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	2019.51±110.88		0.00	2112.49±42.97	
MRS	0.00	1566.88±42.94	-22.41	0.00	1678.46±109.02	-20.55
L58/1	0.00	1121.96±129.88	-28.39	0.00	942.67±75.21	-43.84
L 9/7	1343.37±59.2	1417.75±56.89	-9.52	1265.47±74.7	1664.07±128.46	0
B52	708.98±62.56	1191.09±208.79	-23.98	693.19±27.13	1005.12±93.62	-40.12

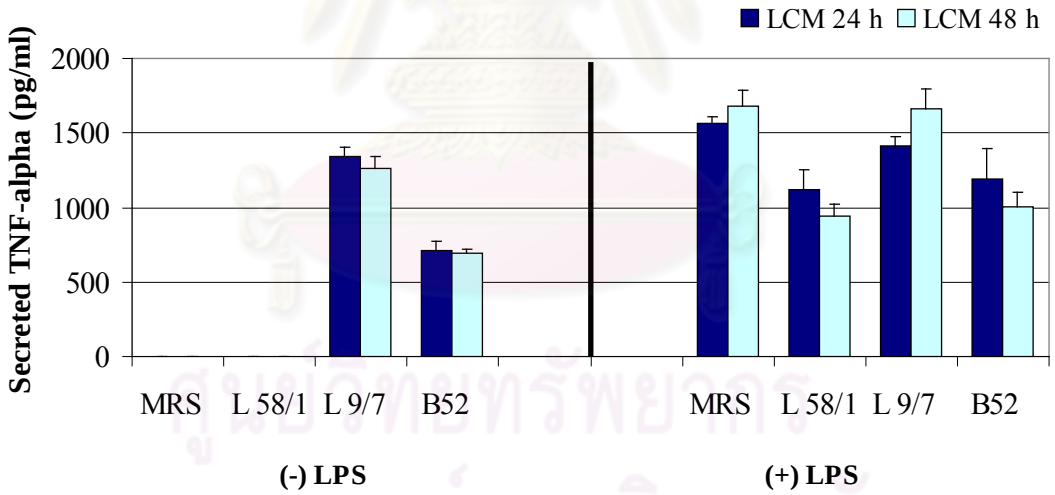


Figure 76. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 69. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1491.12±21.60		0.00	1544.21±71.31	
MRS	0.00	960.64±141.83	-35.58	0.00	925.88±155.76	-40.04
L58/1	0.00	410.64±210.27	-57.25	0.00	358.26±30.02	-61.31
L 9/7	963.02±73.6	1090.4±169.50	13.51	990.29±5.56	1127.07±35.74	21.73
B57	0.00	640.88±168.67	-33.29	0.00	394.93±32.73	-57.35
B84	975.64±32.51	1251.36±74.00	30.26	862.55±42.24	1033.26±85.96	11.60

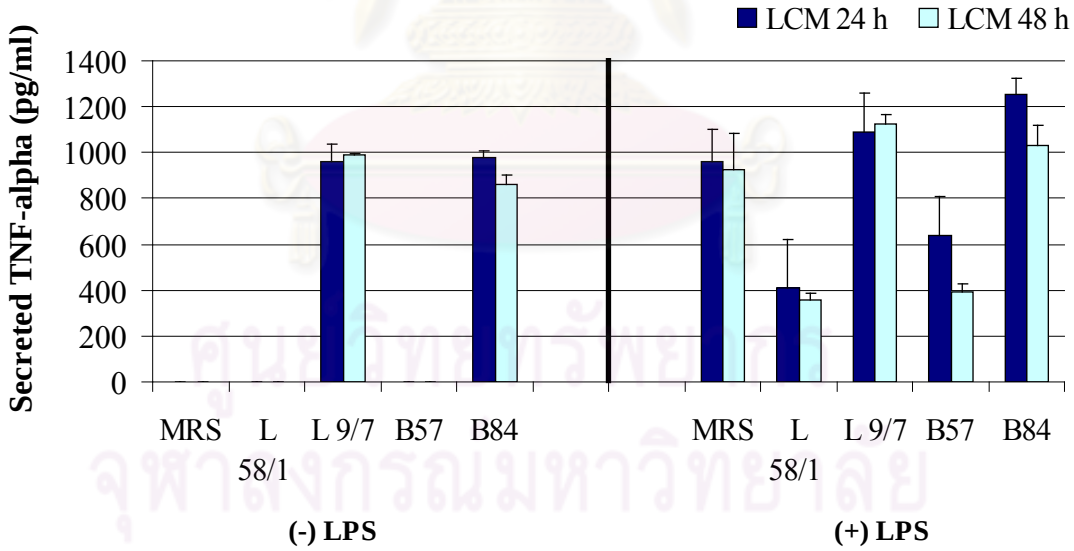


Figure 77. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 70. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1839.27±26.64		0.00	1831.97±112.56	
MRS	0.00	1501.81±202.95	-18.35	0.00	1417.68±65.52	-22.61
L 58/1	0.00	1144.03±128.52	-23.82	0.00	1025.94±3.97	-27.63
L 9/7	1300.54±59.9	1688.16±66.05	12.41	1351.02±11.3	1511.97±70.25	6.65
B61	893.56±63.68	1634.51±67.05	8.84	789.43±77.32	1473.87±84.85	3.96
B62	541.49±11.83	1509.43±71.85	0.51	858±68.10	1315.14±283.85	-7.23

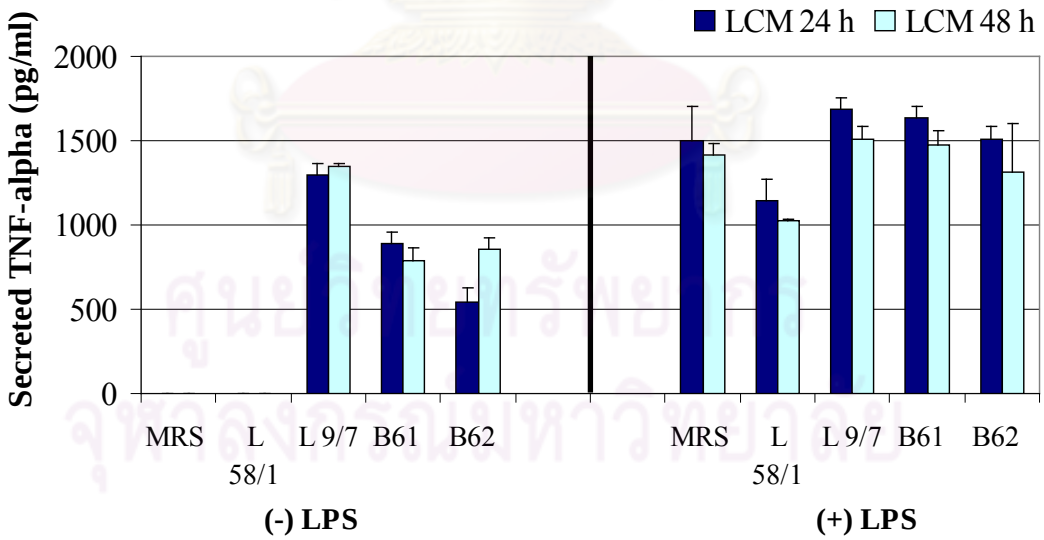


Figure 78. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 71. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer on TNF production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; RPMI, THP-1 media control; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain; SD, standard deviation; % inhibition (-) indicates inhibited TNF-α.

No.	24 h LCM	24 h LCM +LPS	% Inhibition	48 h LCM	48 h LCM +LPS	% Inhibition
	TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD		TNF-α (pg/ml)±SD	TNF-α (pg/ml)±SD	
RPMI	0.00	1886.93±62.56		0.00	1915.93±88.79	
MRS	0.00	1193.93±58.35	-36.73	0.00	1017.93±149.72	-46.87
L 58/1	0.00	848.6±96.06	-28.92	0.00	582.27±192.60	-42.80
L 9/7	1213.6±29.61	1460.93±110.96	22.36	1206.1±28.99	1403.6±108.43	37.89
B85	605.27±32.08	1085.6±140.30	-9.07	756.1±166.17	924.27±88.22	-9.20
B99	324.6±24.25	1272.6±83.47	6.59	414.1±95.46	1116.6±341.13	9.69

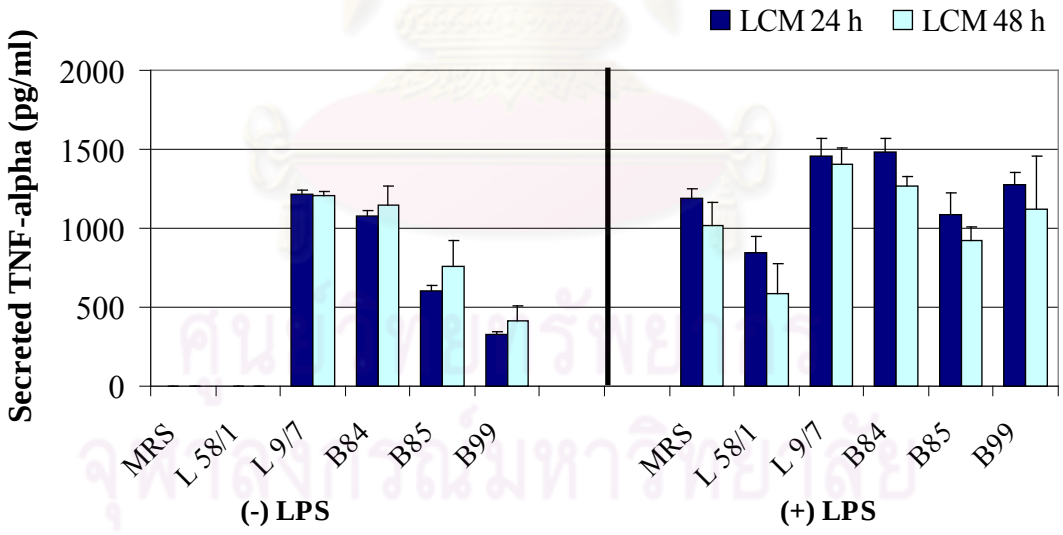


Figure 79. Immunomodulatory effects of *Lactobacillus* isolates from group 3 patients with peptic ulcer patients on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; error bar showed that standard deviation; n=3.

Table 72. Summary of immunomodulatory effects of 24 *Lactobacillus* isolates from group 3 patients with peptic ulcer (16 subjects) on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media;LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; % inhibition , - indicated inhibition

Subject And Patient	**LCM+LPS 24h**	**% TNF-α inhibition**	**P-value**	**LCM+LPS 48h**	**% TNF-α inhibition**	**P-value**
Positive control	L 58/1	-38.20	<0.005	L 58/1	-42.8	<0.05
Negative control	L 9/7	17.52	-	L 9/7	28.43	-
1 (28)	B4/2 B5	-6.26 2.02	- -	B4/2 B5	4.59 17.48	- -
2 (27)	XB7	-77.26	<0.0005	XB7	-57.23	<0.001
3 (57)	B14	7.70	-	B14	11.65	-
4 (67)	B15 B16	42.35 -6.29	- -	B15 B16	30.91 18.38	- -
5 (76)	B23 B24	-12.06 8.10	- -	B23 B24	-39.62 14.74	<0.01 -
6 (85)	B26	-2.66	-	B26	15.39	-
7 (99)	B32 B33	-11.28 0.82	- -	B32 B33	-4.31 10.41	- -
8 (108)	B36 B37	-62.76 -17.79	<0.05 -	B36 B37	-16.10 -25.86	- -
9 (109)	XB40	-53.23	<0.05	XB40	-82.41	<0.005
10 (123)	B43 B44	0 15.02	- -	B43 B44	-18.33 14.20	- -
11 (146)	B52	-23.98	<0.05	B52	-40.12	<0.001
12 (156)	B57	-33.29	<0.05	B57	-57.35	<0.05

Table 72. Summary of immunomodulatory effects of 24 *Lactobacillus* isolates from group 3 patients with peptic ulcer (16 subjects) on TNF-α production in LPS-stimulated THP-1 monocytic cells. LCM, *Lactobacillus* conditioned media; LPS, lipopolysaccharide; MRS, bacterial media control; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non- TNF-α inhibitory strain; % inhibition , - indicated inhibition. (Continued)

Subject And Patient	LCM+LPS 24h	% TNF-α inhibition	P-value	LCM+LPS 48h	% TNF-α inhibition	P-value
13 (158)	B61	8.84	-	B61	3.96	-
	B62	0.51	-	B62	-7.23	-
14 (206)	B84	30.26	-	B84	11.60	-
	B85	-9.07	-	B85	-9.2	-
15 (250)	B98	18.52	-	B98	8.15	-
16 (257)	B99	6.59	-	B99	9.69	-

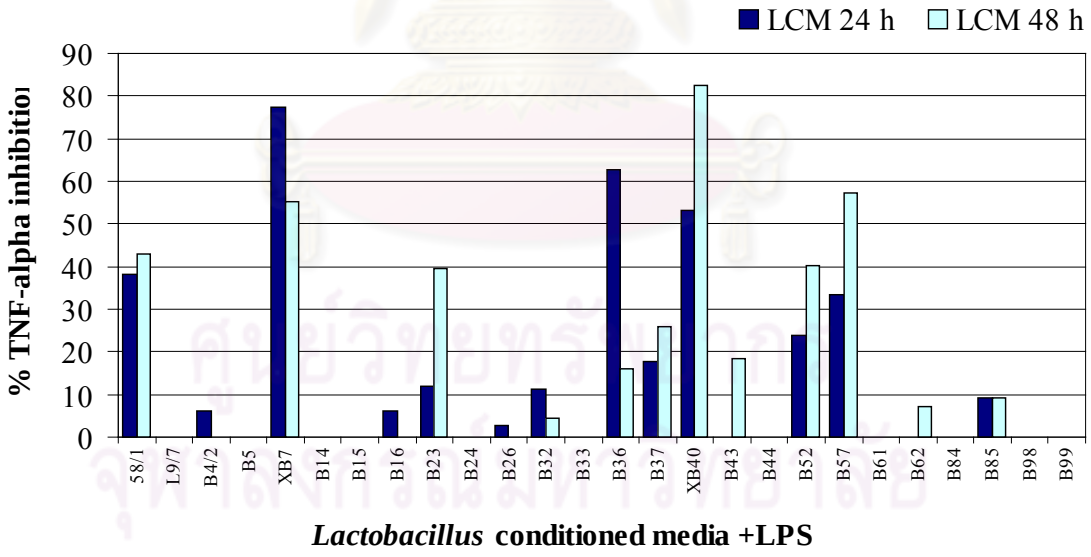


Figure 80. Summary of immunomodulatory effects of 24 *Lactobacillus* isolates from group 3 patients with peptic ulcer (16 subjects) on TNF-α production in LPS-stimulated THP-1 monocytic cells. LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain.

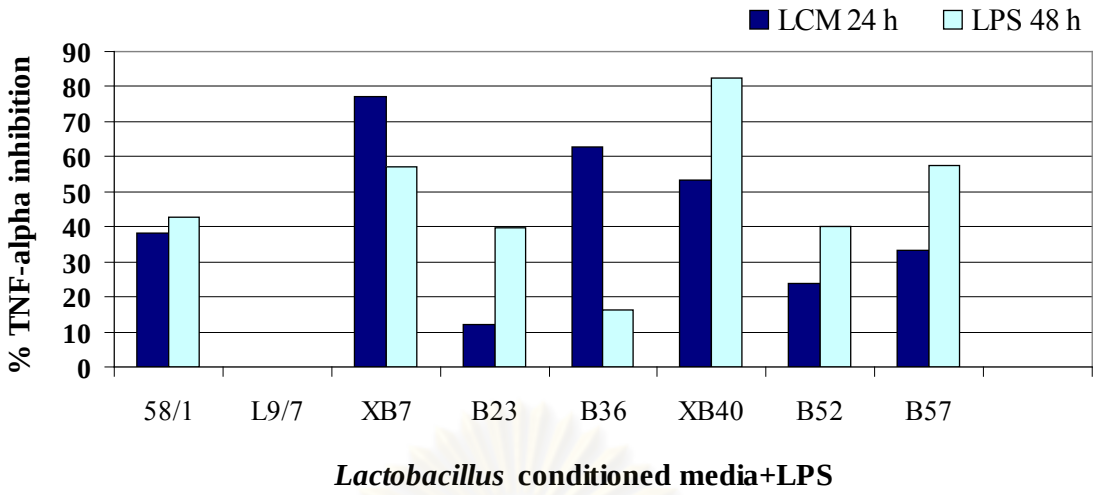


Figure 81. Summary of inhibitory effects of 6 *Lactobacillus* isolates from group 3 patients with peptic ulcer (6 subjects) of TNF-α production by LPS-stimulated THP-1 monocytic cells. LPS, lipopolysaccharide; L 58/1, positive control of TNF-α inhibitory strain; L 9/7, negative control of non-TNF-α inhibitory strain.

Table 73. Species of *Lactobacillus* isolates with TNF-α inhibitory activity from group 3 patients with peptic ulcer.

Colony appearance	**Code**	***Lactobacillus* species**	**Immunomodulatory activity**
M-white turbid	B4/2	*L. salivarius*	Non-TNF-α inhibitory activity
M-white (transparent)	B5	*L. fermentum*	Non-TNF-α inhibitory activity
M-white turbid	XB7	*L. plantarum*	TNF-α inhibitory activity
M-white transparent	B14	*L. fermentum*	Non-TNF-α inhibitory activity
M-white transparent	B15	*L. mucosae*	Non-TNF-α inhibitory activity
M-white turbid	B16	*L. salivarius*	Non-TNF-α inhibitory activity
M-turbid	B23	*L. salivarius*	TNF-α inhibitory activity
M-transparent	B24	*L. fermentum*	Non-TNF-α inhibitory activity
L-white transparent	B26	*L. mucosae*	Non-TNF-α inhibitory activity
M-white turbid	B32	*L. salivarius*	Non-TNF-α inhibitory activity
M-turbid	B33	*L. fermentum*	Non-TNF-α inhibitory activity
M-round turbid	B36	*L. agilis*	TNF-α inhibitory activity
M-round turbid	B37	*L. salivarius*	Not significantly inhibitoty TNF-α activity
M-white transparent	XB40	*L. gasseri*	TNF-α inhibitory activity
M-turbid	B43	*L. salivarius*	Not significantly inhibitoty TNF-α activity
M-transparent	B44	*L. fermentum*	Non-TNF-α inhibitory activity
M-white turbid	B52	*L. salivarius*	TNF-α inhibitory activity
M-white	B57	*L. murinus*	TNF-α inhibitory activity
M-white transparent	B61	*L. fermentum*	Non-TNF-α inhibitory activity
L-round turbid	B62	*L. salivarius*	Non-TNF-α inhibitory activity
M-transparent	B84	*L. fermentum*	Non-TNF-α inhibitory activity
S-turbid	B85	*L. salivarius*	Non-TNF-α inhibitory activity
M-white transparent	B98	*L. fermentum*	Non-TNF-α inhibitory activity
M-transparent	B99	*L. fermentum*	Non-TNF-α inhibitory activity

Table 74. The prevalence of *Lactobacillus* and number of isolates which significantly inhibited TNF-α production by THP1-monocytic cells.

Group of patient (number)	**Number of patients from whom TNF-α –inhibiting *Lactobacillus* was isolated (Prevalence)**	**Number of TNF-α - inhibiting *Lactobacillus* isolates / Number of total isolates**
Mild gastritis (n=9)	7 (77.78%)	10 /16 (62.5%)
Severe gastritis (n=32)	18 (56.25%)	22/47 (46.81%)
Peptic ulcer (n=16)	6 (37.5%)	6 /24 (25%)
Total (n=57)	31 (54.39%)	38/87 (43.68%)

Table 75. Comparison of the prevalence of TNF-α – inhibiting *Lactobacillus* isolated from each group of dyspeptic patients. Statistical values were calculated using the Chi square and multivariate analysis which were considered significant at p-value ≤ 0.05.

Group of patient	**The prevalence of TNF-α- inhibiting *Lactobacillus***	
	Chi-square test	**Multivariate analysis**
Mild gastritis v.s. Severe gastritis	Not significantly different (P = 0.242)	Not significantly different (P=0.917)
Severe gastritis v.s. Peptic ulcer	Not significantly different (p = 0.221)	-
Mild gastritis v.s. Peptic ulcer	Significantly different (p = 0.053)	Not significantly different (P=0.985)

CHAPTER VI

DISCUSSION

The microflora of human gastrointestinal tract contains diverse populations of bacteria which play an essential role in the development of gut mucosal barrier and innate immunity. *Lactobacillus* is commonly associated with the body of humans and animals. They are microflora in the oral cavity, gastrointestinal tracts and vagina (33, 34). Members of *Lactobacillus* are gram-positive, facultatively anaerobic, catalase-negative and non-spore-forming rods and are isolated from many habitats (202).It has been reported that the indigenous lactobacilli were only the species *L.crispatus*, *L. gasseri*, *L. reuteri*, *L.ruminis* and *L. salivarius* (35). Other *Lactobacillus* species found in human were considered transient lactobacilli.

In this study, *Lactobacillus* was isolated from gastric biopsies and throat swabs of dyspeptic patients that were divided into three groups based on endoscopic findings. The patients were starved overnight before the gastroduodenal endoscopy for easy visualization and diagnosis by endoscopist. The prevalence of *Lactobacillus* isolates from gastric biopsies found in patients group 1 and 2 were not significantly different ($p>0.05$). Surprisingly, the prevalence of *Latobacillus* isolates found in patients group 1 and 2 were less than those in patients group 3 significantly ($p<0.05$) as shown in tables 15 and 16. However, the prevalence of *Lactobacillus* isolates from throat swabs of each group of patients were not significantly different ($p>0.05$) as shown in Tables 17 and 18. These results suggested that the stomach environment of peptic ulcer patients was more favourable to *Lactobacillus* than that of patients with mild gastritis and severe gastritis. The human stomach was an inhospitable environment for microorganism because of acidic conditions (pH 2.2-2.4) and other antimicrobial factors (69).

In this study, most *Lactobacillus* isolates were vancomycin resistant. Previous report classified *Lactobacillus* by using vancomycin susceptibility test into vancomycin-resistant and vancomycin-susceptible groups (32). *L. acidophilus* complex comprised of species *L. acidophilus*, *L. johnsonii*, *L. crispatus*, *L. amylovorus*, *L. gallinarum* and *L. gasseri* (33, 147, 203) . *L. acidophilus* group and *L. delbruckeii* were susceptible to vancomycin while *L. rhamnosus* was resistant to vancomycin (171). Most of

vancomycin- susceptible isolates were *L. gasseri* which would be missed if we selected only vancomycin –resistant colonies.

Identification of lactobacilli was previously based on culture-dependent methods and recently, molecular techniques involving gene sequencing were the gold standard. In present study, *Lactobacillus* group-specific primers could not separate *Weissella* from genus *Lactobacillus*. B71 and B59 isolates from gastric biopsies and T117/1, T126, and T127 isolates from throat swabs were amplified by *Lactobacillus* primers (data not shown) and identified by DNA sequencing as *W. confusa* or *W. cibaria* and *W. cibaria*, respectively. This was consistent with the result previously described that these group-specific primer were able to detect of *Lactobacillus, Pediococcus, Leuconostoc*, and *Weissella* (115, 179). Universal primers 16S-8F and 16S-1541R amplified the whole 16S rRNA gene about 1,520 bp long which constituted of both variable and conserverd region (32). The 16S rRNA gene product is large enough and sufficient to distinguish the species of *Lactobacillus*. The universal primer was used carefully because it could amplify the contaminate DNA. For some lactobacilli such as *L. casei* group, the sequencing could not discriminate species identification. Several previously described *L. casei* related strains such as *L. casei*, *L. paracasei*, *L. rhamnosus* and *L. zeae* which classification and nomenclature of these bacteria are controversial. The differences in the V1 region of the 16S rRNA were observed for the three *casei*-group species as *L. casei*, *L. paracasei* and *L. rhamnosus* (204) and reported sequence signatures from the 16S rRNA which allowed differentiation of these species (202). The sequence of *L. casei* group is different within the V1 region of the 16S rRNA gene and polymerase chain reaction primers were designed variable region of each *Lactobacillus* when used combination with primer Y2 (conserved 16S rRNA) enabled amplification of a specific of each strains (205). Moreover, Random amplification of polymorphic DNA (RAPD) analysis was used for strain comparison which specific amplification fragments enabled a rapid presumptive identification of the *Lactobacillus* species (205).

In our study, the data revealed that *Lactobacillus* isolated from throat were presented in various species than *Lactobacillus* isolated from gastric biopsies. The stomach contributed to highly acidic hydrochoric acid (HCL) which destroys most oral bacteria and anaerobic bacteria. Relatively few *Lactobacillus* species can tolerate acidic condition of stomach (28). The gastric biopsies of dyspeptic patients were recovered by

several *Lactobacillus* species, with *L. fermentum* and *L. salivarius* being the predominant species. *L. gasseri*, *L. plantarum*, *L. mucosae* and *L. casei* group can recovered from gastric biosies. Moreover, *L. oris*, *L. murinus* and *W. confusa* or *W. cibaria* few isolated from gastric biopsies. These were consistent with the previous results demonstrating the bacterial diversity within the human gastric mucosa (70). In addition, isolation of *Lactobacillus* from human gastric mucosa obtained from healthy individuals revealed a number of *Lactobacillus* species including novel species as *L. gastricus* sp. nov., *L. antri* sp. nov., *L. kalixensis* sp. nov. and *L. ultunensis* sp. nov. (35).

In this study, the species most frequently recovered from the throat as well as the stomach of dyspeptic patients were *L. fermentun* and *L. salivarius*. Previous reported, *Lactobacillus* microflora isolated from the rectal as well as the oral mucosa of healthy volunteers were *L. plantarum* and *L. rhamnosus* and *L. paracasei* which were isolated from 52%, 26% and 17% of the individuals (34). Most of the major *Lactobacillus* groups, including *L. fermentum* and *L. salivarius* were found both on the throat and stomach. Previous has been suggested that the species *L. salivarius*, *L. crispatus*, *L. gasseri*, *L. reuteri* and *L. ruminis* are truly autochthonous (indigenous microflora) to the human gastrointestinal tracts (33). Inaddition, *L. gasseri* was found in gastric biopsies more than throat swab of dyspeptic patients.

Tumor necrosis factor-alpha (TNF-α) is proinflamatory cytokine and widely appreciated as a principal mediator of systemic responses to sepsis and injury (206). It has been involved in major mediator of inflammation and in the pathogenesis of a wide spectrum of human diseases, including sepsis, diabetes, cancer, osteoporosis, allograft rejection and autoimmune diseases such as multiple sclerosis (20), rheumatoid arthritis, and inflammatory bowel diseases (21, 22). TNF-α is potent inducer of inflammation and expressed in increased amount of mononuclear cell infiltrated into a site of infection. TNF-α is produced by different cell types and produced mainly by activated macrophages in response to tissue injury or infection (207). TNF-α induces a cascade of endogenous mediators that direct host immunologic functions (200). While TNF-α as an essential element in host defense, the excessive tissue production of TNF-α can mediate detrimental systemic effects by acutely precipitating a syndrome similar to that of septic shock (208). TNF-α is reported to promote inflammatory cell infiltration by upregulating

leukocyte adhesion molecules on endothelial cells (ECs), activated neutroplile, promot T and B cell proliferation, serve as a chemotactic agent for monocytes (188).

The prevalence of TNF-α-inhibiting *Lactobacillus* isolates from gastric biopsies found in patients groups 1 and 2 and groups 2 and 3 were not significantly different ($p>0.05$). As postulated, the prevalence and number of isolates found in patients group 1 were more than those in patients group 3 significantly ($p=0.053$) as shown in Tables 74 and 75. However, multivariate analysis of the prevalence of TNF-α – inhibiting *Lactobacillus* isolated from group 1 and group 3 was not significant at ($p= 0.985$) as shown in Table 75.

The inhibitory activity varied variously in each strain, which some isolates showed highly TNF-α inhibitory activity while some isolates slightly inhibited TNF-α production *in vitro*, which indicate that the potential of *Lactobacillus* isolates were functionally different. *Lactobacillus* did not have modulatory effect in every species or isolate of the same species, demonstrating that specific immune effects may be species or strain specific (58). We studied *Lactobacillus* conditioned media at 24 and 48 h cutivation of *Lactobacillus*. Some isolates produced immunoregulatory factor at 24 h, but some isolates did as 48 h of cultivation for immunoregulatory factor production. Therefore, it was possible that the time point was optimal for production and secretion of immunoregulatory factor to modulated TNF-α production *in vitro*.

Previous described some gastrointestinal infection and inflammatory conditions, such as acute gastroenteritis, inflammatory bowel disease (IBD), inflammatory cells including monocytes, lymphocytes, were activated and accumulated in lamina propria. These cells secrete excessive inflammatory products such as proinflammatory cytokine, chemokine. TNF-α can induce epithelial cell secrete IL-8 production which *L. reuteri* inhibited the synthesis and secretion of IL-8 induced by activated with TNF-α. Several probiotic mechanisms of action, have been competitive of probiotic with microbial pathogens, antimicrobial activity and suppression of pathogen growth, immunomodulation and/or stimulation of an immune response, development of gut mucosal barrier and induction of T cell apoptosis (164, 209).

Similarly, previous studies suggested that several species of *Lactobacillus* grown in media and secreted immunoregulatory factor into media culture which down-regulated TNF-α production as celled immunomodulins (32, 58). Similary, this study suggested that some *Lactobacillus* isolates as TNF-α inhibitory strains feasibly secreted immunoregulatory factor into *Lactobacillus* conditioned media, which were capable inhibited TNF-α production in LPS-activated THP-1 momocytic cells. The similar result has been shown that lactobacilli recovered from mice without colitis significantly inhibited TNF-α production by LPS-activated macrophages which 29 lactobacilli isolated from mice without colitis, 6 (21%) displayed TNF-α inhibitory effects. In contrast, none of 29 lactobacilli recovered from colitis mice TNF-α inhibitory activity (32). Pena and Versalovic previously described an in vitro assay demonstrating that *L. rhamnosus* GG were able to inhibit TNF-α production in LPS-activated murine macrophages (58). It has been reported that oral administration of a mixture of (VSL#3) as *Lactobacillus* and *Bifidobacterium* in ulcerative colitis patients has effective in preventing flare-ups of chronic pouchitis (210). Holma R *et al*. found that *L. reuteri* R2LC significantly diminished mucosal inflammation in acetic acid induced. (211). These reports supported the role of *Lactobacillus*-mediated immunomodulation in the diseases resulting from inflammation.

CHAPTER VII

CONCLUSTION

The prevalence of *Lactobacillus* isolates from gastric biopsies found in patients group 1 and 2 were similar. However, the prevalence of isolates found in these groups were less than those in patients group 3 significantly ($p<0.05$). The prevalence of *Lactobacillus* of isolates from throat swabs of each group of patients were not significantly different ($p>0.05$). These results suggested that the stomach environment of peptic ulcer patients was more favourable to *Lactobacillus* than that of patients with mild gastritis and severe gastritis.

The prevalence of TNF-α-inhibiting *Lactobacillus* isolates from gastric biopsies found in patients groups 1 and 2 and groups 2 and 3 were not significantly different ($p>0.05$). As expected, the prevalence of isolates found in patients group 1 were more than those in patients group 3 significantly ($p=0.053$) However, multivariate analysis of the prevalence of TNF-α-inhibitory *Lactobacillus* in patients group 1 and 3 was not significantly different ($p=0.985$).

TNF-α – inhibitory *Lactobacillus* found in this study were all isolates of *L. plantarum, L. murinus* and some isolates of *L. salivarius, L.gasseri and L. casei* group. On the contrary, all isolates of *L. fermentum, L. mucosae* and *L. oris* did not suppress TNF-α production. Predominate species found in both gastric biopsies and throat swabs were *L. fermentum* and *L. salivarius.* The majority of patients, from whom *Lactobacillus* spp. were isolated from both gastric biopsies and throat swabs, had at least one isolate of the same species.

The results of this study suggested that some *Lactobacillus* species detected in gastric biopsies originate from throats and *Lactobacillus* species in the stomach might be a factor contributing to the pathogenesis of peptic ulcer.

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APPENDICES

APPENDIX A

METERIAL AND EQUIPMENTS

1. Materials and reagents

MRS agar	(Oxoid, England)
MRS broth	(Oxoid, England)
Glyseral	(Oxoid, England)
Anaerobic gas packge	(MGC, Japan)
Anaerobic indicator	(Oxoid, UK.)
Vancomycin disks (VA 5 ug/disc)	(Oxoid, England)
Brain heart infusion agar	(BHI; oxiod, England)
Spreader	
Thin wall PCR tube (500 µl)	
Barrier tips 20, 100, 200, 1,000 µl	(Neptune, Mexigo)
PCR water	(Roche, Germany)
Distilled Water DNAse, RNAse Free	(Gibco; Invitrogen, UK.)
High Pure PCR Template Preparation Kit	(Roche, USA)
Absolute alcohol	(MERCK, Germany)
Lysozyme (egg white)	(Ameresco)
Primers	(Invitrogen, Hong Kong)
Fast start Taq DNA polymerase	(Roche, Germany)
Hot start master mix	(GE Healthcare illustra, UK)
Ethylene diamine tetraacetic acid (EDTA)	(USB, Cleveland)
Tris (ultrapure)	(Reseach organism, USA)
Boric Acid	(Reseach organism, USA)
Ultrapure™ Agarose gel	(Reseach, USA)
Ethidium bromide	(Amresco, USA)
GeneRuler 100bp DNA Ladder Plus	(Fermentas)
QIAquick PCR purification kit	(Qiagen Inc., USA)
QIAquick gel extration kit	(Qiagen Inc, USA)
THP-1 monocytic cell lines	(ATCC TIB-202, USA)
Conical centrifuge tube: 15, 50 ml	(NUNC, USA)

Sterile serological pipettes (1, 5, 10 and 25ml)	(NUNC, E.U.)
Pipette boy	(Metrix, Japan)
Cellulose acetate filter 0.2 μm pore size filter unit	(Sartorius,Germany)
Syryne	(Nipro, Thailand)
0.2 μm syryne filter (Minisart)	(Sartorius,Germany)
Tissure culture flask (25 cm^2)	(NUNC, Denmark)
96-well flat-bottom tissue culture plates	(NUNCLON D, Denmark)
96 well-microtiter plates (F96 CERT, MAXISORP)	(NUNC, Denmark)
RPMI 1640 medium	(Gibco-Invitrogen, USA)
Fetal bovine serum (FBS)	(Gibco-Invitrogen, USA)
Hemocytometor (Bright line)	(BOECO, Germany)
Counter	(Thailand)
Trypan blue stain 0.4%	(Gibco-Invitrogen, USA)
Lipopolysaccharaide of *E. coli* serotype O127:B8	(LPS; Sigma, Germany)
Human TNF-α Duoset (DY210)	(R&D, USA)
Substrate Reagent Pack (DY999)	(R&D, USA)
Sulfuric acid (H_2SO_4)	(MERCK,Germany)
Isopropanol (2-Propanol)	(MERCK, Germany)
Hydrochloric acid	(MERCK, Germany)
Sodium hydroxide (NaOH)	(MERCK, Germany)
Sodium bicarbonate ($NaHCO_3$)	(Sigma, Germany)
Sadium chloride (NaCl)	(Sigma, USA)
Potassium chloride (KCl)	(Sigma, England)
Hydrochoric acid (HCl)	(MERCK, Germany)
Na_2HPO_4	(SigmaGermany)
KH_2PO_4	(Sigma, Germany)
Tween 20	(Amresco)
Albumin, bovine serum (BSA)	(Sigma, USA)
Cryovial	(NUNC, Denmark)
Dimethyl Sulfoxide (DMSO)	(Sigma-Aldrich, USA)

2. EQUIPMENT

Microcentrifuge tube	(Eppendorf, USA)
Plastic plate (90mm)	(Millionant, Thailand)
Ultrasonic water bath	(GEN-PROBE, Germany)
Anaerobic chamber	(Concept Plus)
Refrigerated centrifuge	(Sanyo, Japan)
Fireboy	(IBS, Switzerland)
Incubator	(Memmert)
-20°C Freezer	(Sanyo, Japan)
-80 °C Freezer	(Sanyo, Japan)
Hotplate	(Tekstir® Hot plate)
Thermometer	(UK.)
Water bath	(Gyromax TM 929, USA)
Authoried Thermal Master cycler gradient	(Germany)
Electrophoresis	(Wealtec, Taiwan)
Power supply	(ELITE 300 plus, USA)
UV transillumination	(Bio-Rad)
Centrifuge	(Kubota, Japan)
Centrifuge (RC3C)	(Sorvall instrucments)
Light microscope	(Olympus, Japan)
Inversted microscope	(Olympus, Japan)
Safety cabinet	(Augustin, Thailand)
Vertical Laminar Flow workstation	(Microflow, UK.)
Liquid nitrogen (-196^{o}C)	(Taylor-Wharton, USA)
CO_2 incubator	(BINDER, Germany)
Spectrophotometer	(Bio-Rad Smart Spec^{TM} Plus)
Speed-vacuum drying	(Savant instrucments,USA)
Auto pipette: P-10, P-20, P-200, P-1000	(Gilson, France)
Auto pipette: P-10, P-20, P-200, P-1000	(Socorex, Switzerland)
Muti-chanal pipette	(Socorex, Switzerland)
Tip 10, 200, 1000 µl	
Filter flask	(Satorius, Germany)
pH meter	(Thermo scientific, Singapore)

3. Software and program

- Multalin program (http://bioinfo.genotoul.fr/multalin/multalin.html)
- Sequence mach program of the Ribosomal Database Project II (RDP-II; http://rdp.cme.msu.edu)
- GeneBank DNA database search (www.ncbi.nlm.nih.gov/BLAST)
- Microsoftexcel of set trandard curve and calculate concentration of TNF-α

APPENDIX B

MEDIA, SOLUTION FOR CULTURE METHOD

1. deMan, Rogosa and Sharpe (M.R.S.) agar

Typical Formulation		
Peptone mixture	18.0	g/L
Yeast extract	4.0	g/L
Glucose	20.0	g/L
Tween 80	1.0	g/L
Di-potassium hydrogen phosphate	2.0	g/L
Tri-ammonium citrate	2.0	g/L
Sodium acetate anhydrous	3.0	g/L
Magnesium sulphate 7H2O	0.2	g/L
Manganese sulphate anhydrous	0.034	g/L
Agar	12.0	g/L
Distilled water	1,000	ml

pH approximally 6.2 ± 0.2

Suspend by swirling 62.25 g of medium powder in 1,000 ml of distilled or deionised water. The medium was sterized by autoclaving at 121ºC at 15 pounds/inch^2(p.s.i.) for 15 minutes. The sterile medium was cooled at 50ºC and dispensed 20 ml per 90 mm petri dise. Cooled and stored at 4ºC until used.

2. MRS broth

MRS medium	52	g/L
Deionised water	1,000	ml

Suspend by swirling 52 g of medium powder in 1,000 ml of distilled or deionised water. The medium stock was aliqusted 10 ml in conical tube 15 ml.The MRS medium was sterized by autoclaving at 121ºC at 15 pounds/inch^2(p.s.i.) for 15 minutes. The sterile medium was cooled and stored at 4ºC until used.

3. 20 % MRS glycerol stock solution

MRS medium	52	g
Glyseral	200	ml
Distilled water	1,000	ml

Suspend by swirling 6.23g of MRS medium powder in 80 ml of distilled water and plus 20 ml of glycerol (80:20) as 20% MRS glycerol stock. The medium stock was aliqusted 1 ml in cryovial. The medium stock was sterized by autoclaving at 121ºC at 15 pounds/inch ^{2} (p.s.i.) for 15 minutes. The sterile medium was cooled at 50ºC and stored at 4ºC until used.

4. Catalase test (culture identification)

Catalase is an enzyme that splits hydrogen peroxide into water and oxygen. Hydrogen peroxide is a by-product of respiration and is lethal if it accumulates in the cell of organism. Catalase enzyme that can degrade the hydrogen peroxide in the cell before it can do any cell damage. It catalyzed the H_2O_2 to free oxygen (bubbles) and water.

Solution: 3% hydrogen peroxide

5. 0.85% Normal saline solution (suspending of bacterial)

Sodium chloride (NaCL)	8.5	g/L
Distilled water	1,000	ml

Dissolve 8.5g of NaCL in 1,000 ml of distilled water. The saline solution was aliqusted 5 ml in glass tube. Sterized by autoclaving at 121ºC at 15 pounds/inch^2(p.s.i.) for 15 minutes and stored at room temperature until used.

APPENDIX C

REAGENS AND PREPARATIONS

1. 0.5 M Ethylene diamine tetraacetic acid (EDTA),pH 8.0

Dissodium ethylene diamine tetraacetate.$2H_2O$	186.1	g
DDW	800.0	ml
Adjust pH to 8.0		
Adjust volume to 1,000 ml		

Dissolve 186.1 g of EDTA in 800 ml. Stired on magnetic stirrer for adjust pH 8.0 with NaOH and when ensure EDTA dissolved and adjust volume to 1,000 ml. The solution was sterized by autoclaving at 121°C at 15 pounds /inch^2(p.s.i.) for 15 minutes and stored at room temperature until used.

2. 5X Tris-Boric Acid-EDTA (TBE)

225 mM Tris-base	54.0	g
225 mM boric acid	27.5	g
5 mM EDTA, pH 8.0	20.0	ml

Dissolve 54 g of Tris-base and 27.5 g of boric acid in 500 ml of ddH_2O. Add 20 ml of 0.5M EDTA stock and adjust the volume to 1,000 ml

3. 10 mg/ml Ethidium bromide

Ethidium bromide	1.0	g
DDW	100.0	ml

Stir on magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminium toil or transfer to dark bottle and stored at 4°C

4. 1.0% Agarose gel

Agarose (ultrapure)	0.2	g
0.5X TBE	20.0	ml
10 mg/ml Ethidium bromide	1.0	µl

Dissolve 0.2 g of agarose in 20 ml of 0.5X TBE. The solution was melted by using microwave and when gel dissolved and cooled at 50ºC. The gel solution was added with 1µl of ethedium bromide and poured in tray and cooled abut 20 min unit used.

APPENDIX D

MEDIA, SOLUTION FOR TISSUE CULTURE, BIOASSAY AND SANDWICH ELISA METHOD

1. RPMI 1640 medium

RPMI 1640	1	wrap
$NaHCO_3$	2	g
DI	1,000	ml

Measured 2 g of $NaHCO_3$ and dissolved RPMI 1640 and in 1,000 of deiornize-distilled water mixed, adjust pH 7.0 and filted by using 0.2 μm for sterile medium and aliqused in bottle 250 ml and stored in 4°C. When wound using added with 10% of fetal bovine serum.

2. 5X phosphate buffer saline (PBS) pH 7.2

NaCl	40.03	g
KCl	1.006	g
Na2HPO4	5.750	g
KH2PO4	1.021	g
Distilled water	1000	ml

Dissolve the components above in 1,000 ml of distilled water and adjected pH 7.2-7.4 by using NaOH or HCl. The solution was sterized by autoclaving at 121ºC at 15 pounds/inch^2(p.s.i.) for 15 minutes. The solution was filtered with 0.2 μm and stored until used.

3. Reagent Diluent (RD)

Bovine Serum Albumin	1	g
PBS pH 7.2-7.4	100	ml

Reagent Diluent was prepared 1 g Bovine Serum Albumin (BSA) in 100 ml of PBS, pH 7.2 - 7.4 (1% BSA) and filtered by 0.2 μm and stored in 4°C until used.

4. Stop solution (2N H_2SO_4)

20 N H_2SO_4	10	ml
Sterile distilled water	90	ml

Added 10 ml of 20 N H_2SO_4 into 90 ml distilled water with slowly in lamina flow hood which working concentration as 2N H_2SO_4 and stored in glass bottle until used.

Human TNF-α DuoSet (DY210, USA)

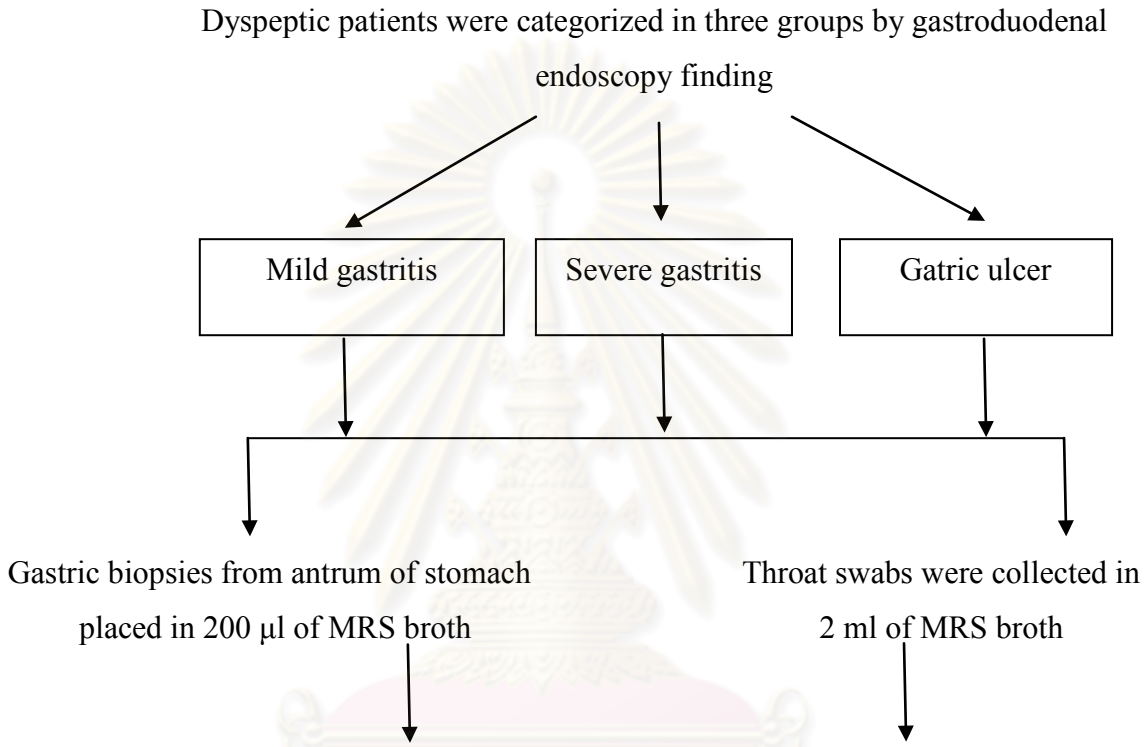
Preparation and storage buffers, diluents, substrates, and solutions of ELISA

1. Detection Antibody (DA) concentration 45 mg/ml of biotinylated goat anti-human TNF-α were reconstituted with 1.0 ml of Reagent Diluent. DA were diluted to a working concentration 250 ng/ml with Reagent Diluent
2. Recombinant human TNF-α (rhTNF-α) concentration 290 ng/ml were reconstituted with 0.5 ml of Reagent Diluent and prepared to 10,000 pg/ml, aliquot and were store at -70° C for set seven point standard curve by use 2-fold serial dilutions in Reagent Diluent and a high standard of 1000 pg/ml is recommended.
3. Streptavidin-HRP concentration 1.0 ml of streptavidin conjugated to horseradish-peroxidase. Before were diluted in the bottle concentrate 1: 200 in Reagent Diluent. The substrate for Streptavidin-HRP is hydrogen peroxide. Cleavage of hydrogen peroxide is coupled to oxidation of a hydrogen donor which changes colure during reaction.
4. Substrate Solution were mixed 1:1 of Color Reagent A (H_2O_2) and Color Reagent B TMB (3,3',5,5'-tetramethylbenzidine) before use only immediately (R&D Systems Catalog No. DY999).

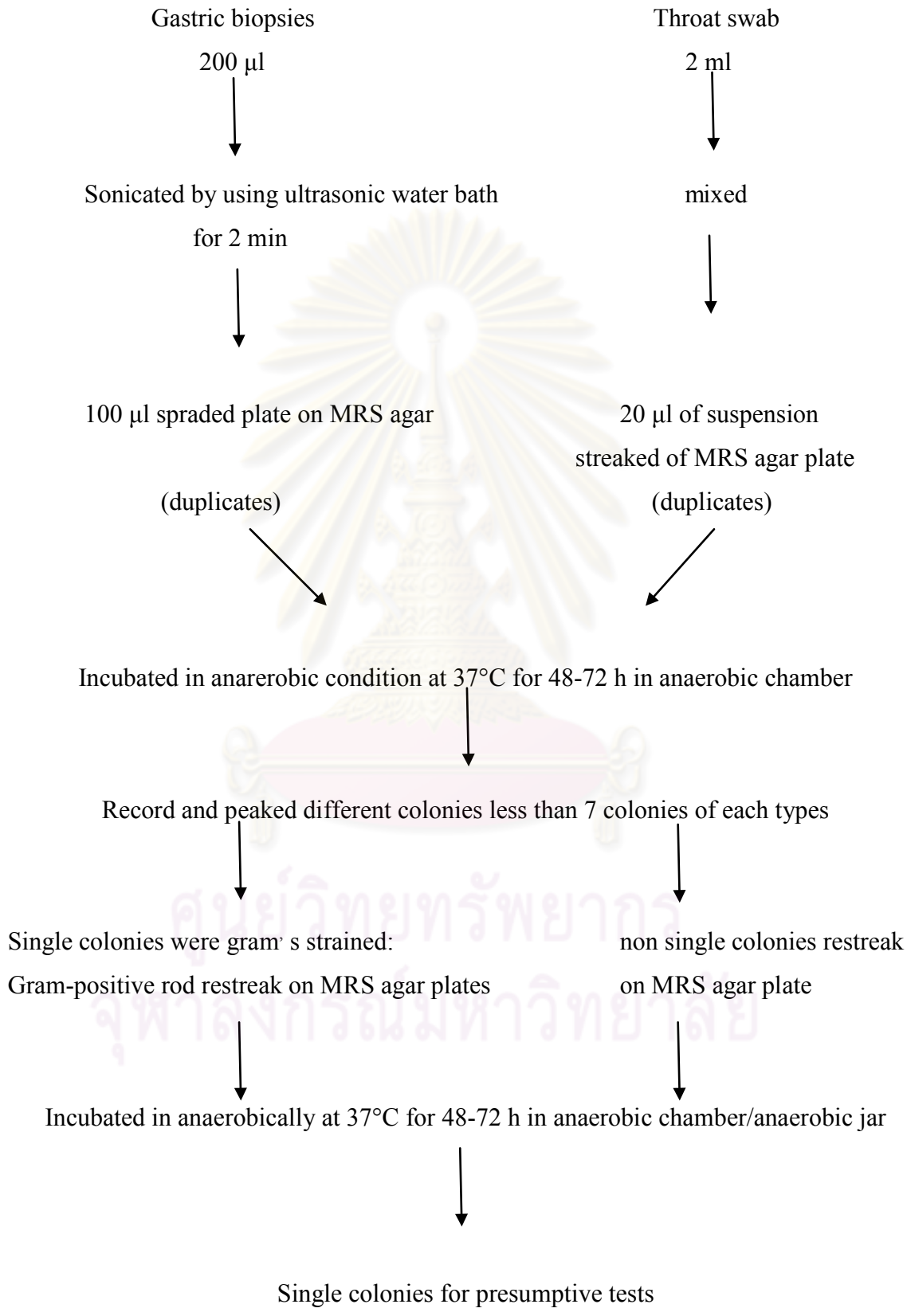
APPENDIX E

FLOW CHART OF PROTOCAL

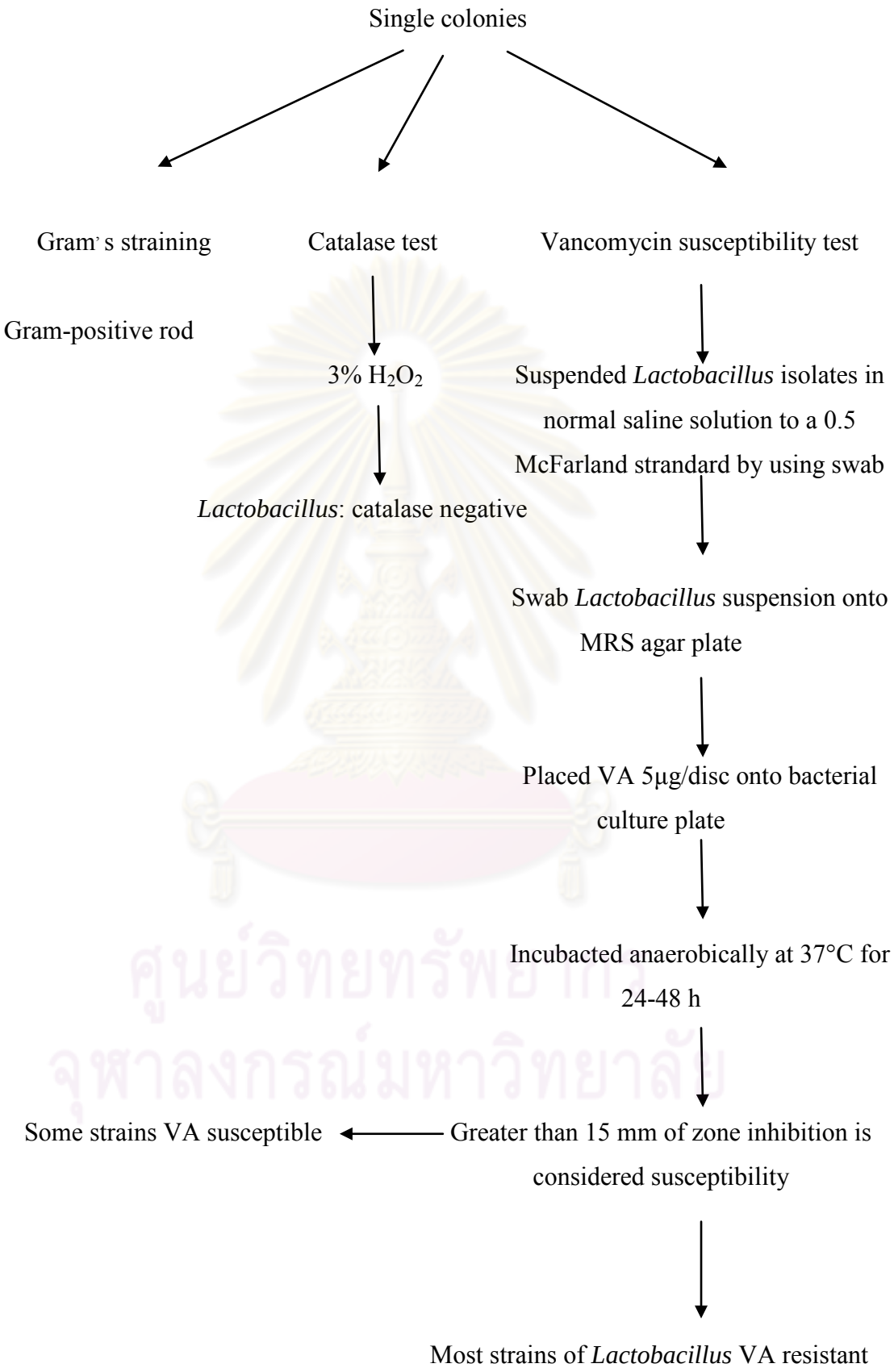
1. Collection of gastric biopsies and throat swab of dyspeptic patients



2. Isolation of *Lactobacillus* from gastric biopsies and throat swab of dyspeptic patients



3. The presumptive test for *Lactobacillus* isolates



4. The preparation of *Lactobacillus* conditioned media (LCM)

Thaw *Lactobacillus* from -80^{o} C freezer stock and recoverated on MRS agar plate

↓

Incubated in anaerobically at 37^{o} C for 24-48 h using anaerobic chamber or in anaerobic jars/boxes

↓

Picked single colony, check pure culture and restreak on MRS agar for isolation

↓

Incubated in anaerobically at 37^{o} C for 24-48 h using anaerobic chamber or in anaerobic jars/boxes

↓

Picked a single colony and incubated into 5 ml of MRS broth in 15 ml centrifuge tubes and grow at 37^{o} C for 24 h

↓

Determined OD_{600} and calculated starting inoculum size of OD_{600} 0.1 in 10 ml of MRS broth in 15 ml centrifuge tubes

↓

Incubated in anaerobically at 37^{o} C for 24 and 48 h using anaerobic chamber or in anaerobic jars/boxes

↓

Centrifuged at 4,000 rpm for 10 min for collected *Lactobacillus* cell-free conditioned media

↓

Filter-sterilized conditioned media with 0.2 μm pore size filter unit

↓

Adjust pH of *Lactobacillus* conditioned media by speed-vacuum drying

↓

Re-suspended *Lactobacillus* conditioned media with aqual volume of RPMI 1640 medium

↓

Stored *Lactobacillus* conditioned media at –20 ^{o}C for until use

5. Stydy immunomodulatory effect of LCM on LPS-stimulated THP-1 monocytic cell lines

Counted and diluted THP-1 cells into fresh culture media to a density $2.5x10^5$ cells/ml

↓

Seeded 200 µl of cell suspension into each well of 96-well microtiter plate

↓

Added 5% (v/v) *Lactobacillus* conditioned media into the appropriate wells

↓

Added 5 µl (final conc.100 ng/ml) of *E. coli* serotype O127:B8 lipopolysaccharaide into the appropriate wells

↓

Incubated in a 37°C, humidified, 5% CO_2 chamber

↓

Centrifuge at 1000 RCF for 10 minutes in 4°C and collected supernatants

↓

Test for cytokine secretion by ELISA (Quantikine TNF-alph/ TNF-SFII human DuoSet)

Test forcell viability using Trypan Blue Dye Exclusion Assay

BIOGRAPHY

Miss Wimonrat Panpetch was born on August 10, 1982 in Krabi, Thailand. She graduated with Bachelor degree of Science in Applied Biology from the Faculty of Science at Suan Dusit Rajabhat University in 2004.