STUDY OF FECAL MICROBIOTA IN HEALTHY INFANT IN UBON RATCHATHANI PROVINCE

JACKSON ALOR MEYIN BELBAK

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE MAJOR IN BIOMEDICAL SCIENCES

COLLEGE OF MEDICINE AND PUBLIC HEALTH
UBON RATCHATHANI UNIVERSITY

ACADEMIC YEAR 2018

UBON RATCHATHANI UNIVERSITY

THESIS APPROVAL

MASTER OF SCIENCE

MAJOR IN BIOMEDICAL SCIENCES

COLLEGE OF MEDICINE AND PUBLIC HEALTH

TITLE STUDY OF FECAL MICROBIOTA IN HEALTHY INFANT IN UBON RATCHATHANI PROVINCE

AUTHOR MR. JACKSON ALOR MEYIN BELBAK

EXAMINATION COMMITTEE

DR. CHAMRAJ KAEWRAEMRUAEN	CHAIRPERSON
ASST. PROF. DR. MARUTPONG PANYA	MEMBER
PROF. DR. PONGSAK RATTANACHAIKUNSOPON	MEMBER
ASST. PROF. DR. SURASAK WANRAM	MEMBER

ADVISORS

..... ADVISOR

(ASST. PROF. DR. MARUTPONG PANYA)

..... CO-ADVISOR

(PROF. DR. PONGSAK RATTANACHAIKUNSOPON)

.....

(DR. NIRUN PHITAKWATCHARA)

DEAN, COLLEGE OF MEDICINE AND PUBLIC HEALTH

.....

(ASSOC. PROF. DR. ARIYAPORN PONGRAT)

VICE PRESIDENT FOR ACADEMIC AFFAIRS

ACADEMIC YEAR 2018

ACKNOWLEDGMENTS

I would like to express my deepest thanks to the Thailand International Cooperation Agency (TICA) for awarded me to study in Thailand. I also appreciate to the TICA as sponsor, which without this, study would not have been achievable. My appreciation to the Ministry of Health Republic of South Sudan for nominated me for this study and allowing me to be away from work for the study.

I would like to express my sincere gratitude to my advisor Assistant Prof. Dr. Marutpong Panya for the continuous support of my study and research, for his patience, motivation, enthusiasm, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my MST study.

Besides my advisor, I would like to thank the rest of my thesis committee: co-advisor Prof. Pongsak Rattanachaikunsopon, ASST. Prof.Surasak Wanram, for their patient, guidance on the bench and all effort to help this study a success. As well as to all wonderful lecturers of College of Medicine and Public Health. And for all the fun we have had in the last two years. Also I thank my friends in Ubon Ratchathani University.

Last but not the least; I would like to thank my family: my parents Alor Meyin and Ateng Deng, for giving birth to me at the first place and supporting me spiritually throughout my life.

JACKSON ALOR MEYIN BELBAK

Researcher

บทคัดย่อ

เรื่อง : การศึกษาไมโครไบโอตาในอุจจาระของเด็กทารกในจังหวัดอุบลราชธานี
ผู้วิจัย : JACKSON ALOR MEYIN BELBAK
ชื่อปริญญา : วิทยาศาสตรมหาบัณฑิต
สาขาวิชา : ชีวเวชศาสตร์
อาจารย์ที่ปรึกษาหลัก : ผู้ช่วยศาสตราจารย์ ดร.มารุตพงศ์ ปัญญา
อาจารย์ที่ปรึกษาร่วม : ผู้ช่วยศาสตราจารย์ ดร.พงศ์ศักดิ์ รัตนชัยกุลโสภณ
คำสำคัญ : Gut Microbiota, Bifidobacterium Groups, Gut composition

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อสำรวจแบคทีเรียสปีชีส์ *Bifidobacterium* sp. และ *Lactobacillus* sp. ในอุจจาระของเด็กแรกเกิดที่เกิดด้วยวิธีธรรมชาติ มีสุขภาพดี และดื่มนมแม่ ผลการศึกษาพบว่าสามารถคัดแยกแบคทีเรียจากตัวอย่างอุจจาระของเด็กอายุ 2 เดือน ได้จำนวน 50 โคโลนี บนจานเพาะเชื้อ MRS agar ที่มีส่วนผสมของแคลเซียมคาร์บอเนต ผลการย้อมสีแกรมพบว่าแบคทีเรียทุกไอโซเลตมีรูปร่างคล้ายอักษรตัววายและรูปร่างท่อน การทดสอบเอนไซม์คะตะเลสให้ผลลบ ซึ่งอาจเป็นไปได้ว่าแบคทีเรียทุกไอโซเลตเป็นเชื้อ *Bifidobacterium* sp. จึงทำการจำแนกเชื้อด้วยเทคนิคพีซีอาร์ด้วยไพร์เมอร์และสภาวะพีซีอาร์ที่จำเพาะต่อเชื้อ *Bifidobacterium* sp. ในสปีชีส์ต่างๆ ได้แก่ *Bifidobacterium infantis Bifidobacterium bifidum Bifidobacterium longum* และ *Bifidobacterium breve* ผลการจำแนกพบว่าทุกไอโซเลตไม่ถูกจัดในสปีชีส์ *B. bifidum* และ *B. longum* เนื่องจากไม่มีแบนดีเอนเอที่จำเพาะเกิดขึ้น สำหรับการจำแนกสปีชีส์ *B. breve* พบดีเอนเอแบนขนาด 288 bp. จากแบคทีเรียไอโซเลต JB1 จึงจำแนกเชื้อเป็น *B. breve* ส่วนการจำแนกสปีชีส์ *B. Infantis* พบว่ายังไม่สามารถจำแนกได้ เนื่องจากมีดีเอนเอแบนที่ไม่จำเพาะเกิดขึ้น ซึ่งจำเป็นต้องมีการปรับสภาวะของพีซีอาร์ให้มีความเหมาะสมต่อไป

ABSTRACT

TITLE : STUDY OF FECAL MICROBIOTA IN HEALTHY INFANT IN UBON RATCHATHANI PROVINE
AUTHOR : JACKSON ALOR MEYIN BELBAK
DEGREE : MASTER OF SCIENCE
MAJOR : BIOMEDICAL SCIENCES
ADVISOR : ASST. PROF. MARUTPONG PANYA, Ph.D.
CO-ADVISOR : PROF. PONGSAK RATTANACHAIKUNSOPON, Ph.D.
KEYWORDS : Gut Microbiota, *Bifidobacterium* Groups, Gut composition

The objective of this study was to investigate the bacterial species of *Bifidobacterium ssp.* and *Lactobacillus ssp.* presented in fecal samples of healthy breastfed infants. The selection of species for study was based on the predominate species in human gastrointestinal tract. Totally, one feces sample was collected from volunteer who was born by cesarean birth. He was two months old of age. Fifty single colonies with clear zone around their colonies were selected from MRS agar medium containing calcium carbonate under anaerobic conditions. Gram staining revealed that all bacterial isolates were gram-positive with a Y-like and bacilli shape, catalase negative, indicated that all these fifty isolates were possibility as *Bifidobacterium* species. For these reasons, the specific primers and PCR condition for identification of *Bifidobacterium s*pecies including *Bifidobacterium* infantis, *Bifidobacterium bifidum*, *Bifidobacterium longum* and *Bifidobacterium breve* were used. The result demonstrated that all bacterial isolates were not *Bifidobacterium bifidum* and *Bifidobacterium* longum, due to the specific band were not detected from genomic DNA sample. For *Bifidobacterium breve*, the bacterial isolates JB1 could show the specific band of 288 bp., indicated that this isolate was belonged B. breve. For identification of *Bifidobacterium infantis*, it was demonstrated that all genomic DNA showed non-specific bands were still presented in the mixture of amplified PCR product. Thus, the PCR conditions are required for optimization.

CONTENTS

	PAGE
ACKNOWLEDGMENT	**I**
ABSTRACT	**II**
CONTENTS	**IV**
LIST OF TABLES	**VI**
LIST OF FIGURES	**VII**
LIST OF ABBREVIATIONS	**VIII**
CHAPTER 1 INTRODUCTION	
1.1 Background and rational of the study	1
1.2 Research question	2
1.3 Hypo thesis	2
1.4 Objective	3
1.5 Scope and limitation	3
1.6 Anticipated outcomes	3
CHAPTER 2 LITERATURE REVIEWS	
2.1 Definition of gut microbiota	4
2.2 Composition of gut microbiota in human gut	4
2.3 Role of gut microbiota in human health	6
2.4 Recent report on human gut microbiota	7
2.5 Bifidobacterium species	8
2.6 *Lactobacillus species*	15
CHAPTER 3 RESEARCH METHODOLOGY	
3.1 Study design.	17
3.2 Ethic permission	18
3.3 Fecal samples collection	18
3.4 Gnomic DNA extraction	18
3.5 Oligonucleotide primers for PCR am plication	18
3.6 PCR component and condition	19
3.7 Gel electrophoresis	21

CONTENTS (CONTINUED)

	PAGE
3.8 DNA analysis	21
3.9 Site for conducting the study	22
CHAPTER 4 RESULTS	
4.1 I solation of Lactic acid bacteria from human feces	23
4.2 Extraction of Genomic DNA from isolated bacteria	24
4.3 Molecular identification of Bacterium species	25
4.4 Specific identification of JB isolates	26
CHAPTER 5 DISCUSSION AND CONCLUSION	**30**
REFERENCES	**33**
CURRICULUM VITAE	**38**

LIST OF TABLES

TABLE		PAGE
3.1	Specific primers based on 16srRNA sequences	18
3.2	PCR condition used for amplification of 16srDNA gene	19
4.1	Quantity and quality of genomic DNA isolated from JB	24

LIST OF FIGURES

FIGURE		PAGE
2.1	Factors which influence the composition of the human gut microbiota	6
2.2	*Bifidobacterium* genome	12
2.3	*Lactobacillus* genome	13
2.4	Maternal and postnatal factors	13
2.5	Infants gut microbiota and host immune system interaction	15
4.1	Gram staining of JB1	23
4.2	Showed product of 16S rDNA gene from *Lactobacillus rhamnosus* NH3. Lane 2 to 9 = amplified product of 16S rDNA gene from JB1 JB2 JB3 JB16 JB18 JB20 JB25 JB27	25
4.3	Ethidium bromide staining gel of amplified products of 16S rDNA of JB isolates with specific primer InfU5 and InfL6	26
4.4	Ethidium bromide staining gel of amplified products of 16S rDNA of JB isolates with specific primer BiBIF-1 and BiBIF-2	27
4.5	Showed the amplification of specific 16S rDNA gene for identification of *Bifidobacterium breve* from isolates JB1, JB and JB5	28
4.6	Ethidium bromide staining gel of amplified products of 16S rDNA of JB isolates with specific primer BiBRE-1 and BiBRE 2	29

LIST OF ABBREVIATIONS

CHARACTER	MEANING
CFU	Colony forming unit
°C	Degree Celsius
DNA	Deoxyribonucleic acid
g	Gram
h	Hours
Kb	Kilobase pairs
µg	Microgram
µL	Microliter
µm	Micrometer
mL	Milliliter
mm	Millimeter
min	Minute
PFU	Plaque forming unit
/	Per
%	Percent
rpm	Revolutions per minute
RNA	Ribonucleic acid
TEM	Transmission electron microscope
$\times g$	Multiple displacement amplification
SDS	Sodium dodecyl sulfate
EDTA	Ethylenediaminetetraacetic acid
TAE	Tris-acetate

CHAPTER 1
INTRODUCTION

1.1 Background and rational of the study

The gut microbiota is defined as the entire microbial population living in human's intestine. These microorganisms include bacteria, fungi, archaea, and protozoans(Jandhyala SM, Talukdar R, Subramanyam C,2015). In human life, the microorganism especially bacteria, is immediately colonized in gastrointestinal tract after birth. It has been demonstrated that the human gut comprises of more than 35,000 bacterial species. Of these species, the species of Bifidobacterium spp. and Lactobacillus spp. that belong to the phylum Actinobacteria and Firmicutes are one of the earliest and most abundant bacterial colonizers of the neonatal or infants gut and are well known to confer a myriad of benefits to the host intestinal, metabolic and immune health (Nagpal R, Kurakawa T, Tsuji H, Takahashi T, Kawashima K, Nagata S, et al ,2017). It has been reported that the early intestinal colonization in neonates, a person whose age of four months, with Bifidobacterium spp. and Lactobacillus spp. can protect the human body from many different types of diseases. This protection is possibly mediated by the beneficial effects of probiotic action such as maintenance of the balance of human normal intestine flora (Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al,2005), modulating the immune response (Yasui H, Ohwaki M,1991), inhibiting pathogen infection by competing the colonization at the mucosal surface, inhibitory effect on colon cancer e.g. by modulation of colonic cell proliferation in rats (Singh J, Rivenson A, Tomita M, Shimamura S, Ishibashi N, Reddy BS, 1997), and protection against virus infection (Duffy LC, Zielezny MA, Riepenhoff-Talty M, Dryja D, Sayahtaheri-Altaie S, Griffiths E, et al, 1994).

In the early colonization is influenced by a many of factors including mode of birth delivery (vaginal or caesarean), diet during infancy (breast milk or formula feeds), antibiotic treatment, and environment exposure, has an effect to the

composition of human's gut microorganism (Murphy K, Curley D, O'Callaghan TF, O'Shea CA, Dempsey EM, O'Toole PW, et al,2017). It was found that the intestinal microbiome composition differing in breast-fed and formula-fed infants. Breast-fed infants have been reported to have a more stable bacterial population, while the microbiota of formula-fed infants appears to be more divers. At the period of breast-fed infant, the populations of *Bifidobacterium* and *Lactobacillus* remain highly abundant in the intestinal microbiota (Davoodabadi A, Dallal MMS et al., 2015; Talarico ST, Santos FE, Brandt KG, et al., 2017)

Infant-type bifidobacterium present during infants induce the immune system towards tolerance during the introduction of ingested antigens. For these reasons, Bifidobacterium levels have been studied in infants across the globe. Interestingly, it seems that not all infants have large amounts of bifidobacteria in their stool. Comparisons of worldwide datasets (Norway, Sweden, Canada, Italy, Switzerland, Bangladesh, the USA, Malawi, and Finland) showed that the gut microbiomes of healthy breast-fed infants in some populations had lower amounts of bifidobacterium than others (Lewis ZT, Mills DA,et al,2017, Turroni F, Peano C, Pass DA, Foroni E, Severgnini M, Claesson MJ, et al, 2012). B. longum species is commonly detected in studies of the infant fecal microbiota.

For the above reasons, this study aims to investigate the two major bacterial genera that include *Lactobacillus* sp. and *Bifidobacterium* sp. in feces of breast-fed infants who was born with vaginal delivery in Ubon Ratchathani Province.

1.2 Research questions

Can the conventional PCR technique is useful for detection of *Bifidobacterium* spp. and *Lactobacillus* spp. in fecal sample of healthy breast-fed infants?

1.3 Hyphosis

Bifidobacterium spp. and *Lactobacillus* spp. Profile can be determined by conventional PCR.

1.4 Objective

To investigate the *Bifidobacterium* spp. and *Lactobacillus* spp. found in fecal samples of healthy breast-fed infants.

1.5 Scope and limitation of research

This study aimed to use of the conventional PCR technique and followed by agarose gel electrophoresis to investigate the bacterial genera in fecal sample derived from infants who were born with virginal delivery mode. The total genomic DNA was extracted from feces by commercial kit .The total number of fecal sample was approximately two samples. The comparative analysis between samples was not determined in this study.

1.6 Anticipated outcomes

To know the two bacterial genera in fecal samples of infant who was born by vaginal delivery and C section delivery.

CHAPTER 2
LITERATURE REVIEWS

2.1 Definition of gut microbiota

The gut micro biota is the wide, complex and diverse collection of microorganisms living in the intestine, including bacteria, archaea and unicellular eukaryote. This microbiota performs various functions and contributes significantly to the digestion and the health of the host also the gastrointestinal microbiota plays a crucial role in health and disease of the host through its impact on nutrition, pathogenesis and immunity and this dynamic microbial population develops rapidly from birth until 2 to 3 years of age or infants period (Hill CJ, Lynch DB, Murphy K, Ulaszewska M, Jeffery IB, O'Shea CA, et al 2017),(Rajilic-Stojanovic M, Smidt H207).

2.2 Composition of gut microbiota in human

Gut bacteria are important components of the microbiota ecosystem in the human gut. Commensal bacterial colonize in the gut shortly after birth and comprise approximate 1000 species, most of which are unknown species belonging to anaerobic strains. The composition and temporal patterns of gut microbiota in infants varies widely and is very different from those in adults (Zhang YJ, Li S, Gan RY, Zhou T,et al,2015). In infants, early acquisition of gut microbiota has been linked to development of innate immune responses and terminal differentiation of intestinal structures. The developing gut microbiota of neonates differs widely between individuals and both internal host properties and external factors influence the establishment of the microbiota. At birth, the infant microbial population resembles the maternal vagina or skin microbiota depending on mode of delivery, i.e. by vaginal delivery or Caesarean section (CS) and also there is other factors have been influence on this development, including antibiotic exposure and breastfeeding. Development of the microbiota occurs as bacteria are replaced in a dynamic, non-random pattern (Kuang YS, Li SH, Guo Y, Lu JH, He JR, Luo BJ, et al,2016).

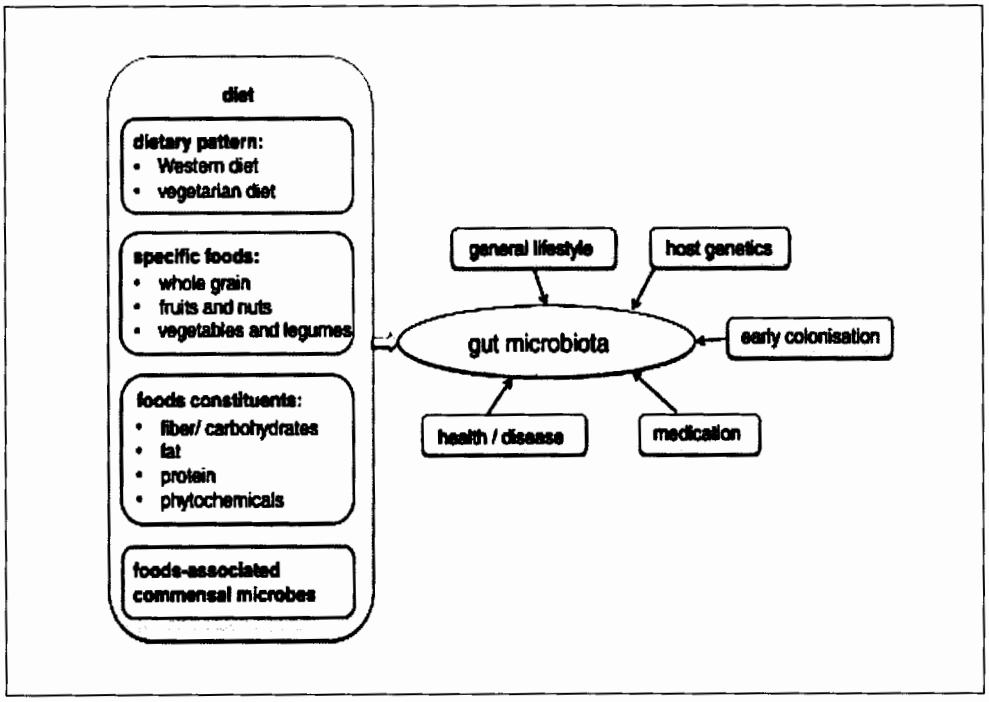

Figure 2.1 Factors, which influence the composition of the human gut microbiota

Source: Graf D, et al. (2015)

2.3 Role of gut microbiota in human

The human gut microorganisms have an impact on both physiology and host health. Well-balanced gut microbiota is very important for good host health. In the GI tract, macronutrients are digested by enzymes and absorbed by cells (Thorsby E, Juge N.2017). However, humans cannot digest fiber present in the food. Gut bacteria use fermentation to help digest dietary fibers that humans cannot break down (Wall R, Ross RP, Ryan CA, Hussey S, Murphy B, Fitzgerald GF, et al,2009). While many bacteria can affect the body negatively, a few kinds are actually essential for well-being. The diverse bacterial species within this ecosystem each contain a battery of enzymes capable of performing a myriad of different functions, ranging from transformation of substances present in the gut (to less or more concentration), the production of antimicrobial substances active against pathogenic bacteria and stimulation of the immune system and also Since colonization with a non-pathogenic microbiota is essential to infant health and probably also has an effect on overall health status in later life, it is important to understand how the composition of this gut microbial ecosystem is established. Moreover, given the importance of the

establishment of a healthy GIT in early life .the kind of microbial, Bifidobacterium, is known to have numerous beneficial of the most important qualities is *Bifidobacterium's* ability to maintain healthy balance of intestinal flora by producing organic compounds. Bifidobacterium is additionally able to restore the population of beneficial bacteria within the body (Khajuria N, Metgud R,2015). Another benefit of Bifidobacterium is its ability to alleviate the symptoms of inflammatory bowel disease. Not only can it alleviate the symptoms of diarrhea, it can often prevent diarrhea from occurring. An additional benefit of Bifidobacterium is that it helps protect the body from urogenital infections. Urogenital tract infection is a disorder that causes a person to go to the bathroom uncontrollably. Another beneficial quality of Bifidobacterium is that it encourages resistance to intestinal infections. A direct benefit of this resistance is that it boosts the entire immune system (Jonkers D, Stockbrugger R.2003). Additionally, the belief that Bifidobacterium has the ability to prevent certain types of cancer by removing toxins from the lower tract and disabling possibly carcinogenic bacteria is held by many scientists. By destroying bad bacteria, normalizing intestinal PH, and restoring the population of beneficial bacteria, Bifidobacterium impacts the body in a beneficial way (Kook SY, Kim Y, Kang B, Choe YH, Kim YH, Kim S,2018). Also the Lactobacillus exhibits a mutualistic relationship with the human body as it protects the host against potential invasions by pathogens, and in turn, the host provides a source of nutrients. Lactobacillus is the most common probiotic found in food such as yogurt, and it is diverse in its application to maintain human well-being as it can help treat diarrhea, vaginal infections and skin disorders such as eczema (Odamaki T, Kato K, Sugahara H, Hashikura N, Takahashi S, Xiao JZ, et al,2016).

2.4 Recent report on human gut microbiota

A recent report suggested that the age-affiliated microbiota population shifts from 3 days to 2 years after birth and that major differences are apparent between 2 years and adulthood (FAO/WHO,2002). Also another report suggested that the host immune defenses along the intestine, including a mucus barrier, help prevent potentially harmful bacteria from causing damage to tissues and also the maintenance of a diverse

and thriving population of beneficial gut bacteria helps to keep harmful bacteria at bay by competing for nutrients and sites of colonization.

2.5 Bifidobacterium species

Classification of the Bifidobacterium is gram-positive, non-spore forming, irregular rod-shaped bacteria that often resemble Y or V shapes. While they are generally classified as obligate anaerobes, which mean they will die when exposed to oxygen, some of the species of Bifidobacterium can tolerate oxygen very well. Bifidobacterium is a micro floral bacterium. Basically, that means that the human body will benefit from this type of bacteria. Bifidobacterium is also considered a probiotic. This type of bacteria is found in the colons of humans. Bifidobacterium got its name from its shape. It is a bifurcated Y shaped rod. Bifidobacterium is an anaerobic bacterium. An anaerobic bacterium is a bacterium that can live its life without the need for oxygen. There are 30 types of Bifidobacterium that are found in many animals. You will usually see an italicized B. in front of the 30 types of Bifidobacterium to list it in that group. It is actually one of the first bacteria that colonize in the large intestine when a person is born. This type of bacteria is used in medicines and dairy to help the immune system grow.

Kingdom: Bacteria

Phylum: *Actinobacteria*

Class: *Actinobacteria*

Subclass: *Actinobacteridae*

Order: *Bifidobacteriales*

Family: *Bifidobacteriaceae*

Genus: *Bifidobacterium*

Bifidobacterium is known to have numerous beneficial qualities. One of the most important qualities is Bifidobacterium's ability to maintain a healthy balance of intestinal flora by producing organic compounds. In this study will focus to detect the three species Bifidobacterium breve, B. bifidium, B. longum.

2.5.1 Bifidobacterium breve

Members of the genus *Bifidobacteria* are considered the most prominent colonizers of the human gastrointestinal tract subsequent to birth, representing ~25% -

80% of the cultivatable bacteria isolated from the feces of infant and adult humans. Members of the species Bifidobacterium breve are anaerobic, rod-shaped, gram-positive bacterium that lack cell motility, sporulation, and a cell capsule. Although B. breve have been found within the gut flora of fully grown adult humans, they are found in much higher quantities within the infant gut and were first isolated from breast-fed infant feces in 1990 . B. breve has unique metabolic capabilities that underlie their importance as a dominant commensal bacterium within the gut. B. breve, in particular, plays a key role in human infant metabolism of breast milk, which contains Human Milk Oligosaccharides (HMO) indigestible by the human host. B. breve is one of three species of the genus Bifidobacterium that demonstrates probiotic capabilities. The particular species B. breve has been studied for its potential in treating childhood constipation in conjugation with Bifidobacterium bifidum and Lactobacillus acidophilus.

2.5.1.1 Structure

The species Bifidobacterium breve has a single circular chromosome consisting of 2,422,684-bp with a relatively high guanine-cytosine (G+C) composition of 58.7%. The genome of B. breve is anticipated to comprise 1,985 genes, 1,854 of which are protein-coding gene sequences estimated to an average length of 1,099 bp. Additionally, the B. breve genome is estimated to encompass an average 1817 Open Reading Frames (ORFs), 26% of which are, after a BLAST-based silicon analysis, expected to encode hypothetical proteins. In a B. breve genome sequencing analysis only one of the 8 analyzed strains was observed to contain a plasmid, thus suggesting infrequent plasmid presence within the B. breve species. B. breves are saccharolytic organisms that depend on carbohydrates present within their host's gastrointestinal tract for their carbon and energy sources.

2.5.1.2 Ecology

Bifidobacterium breve is primarily located in human breast milk and the gastrointestinal tract of infant and adult humans, where they are among the first microbial colonizers, passed from the mother to her offspring. Bifidobacterium breve exhibit a symbiotic relationship with their host by exploiting their unique metabolic capabilities in order to catabolize certain carbohydrates, such as the oligosaccharides present in human breast milk, that are indigestible by their host .As

an individual ages the total population of Bifidobacterium breve within their gut markedly decreases. This could possibly be due in part to the lack of antibiotic resistance exhibited by B. breve, which promotes relatively easy annihilation through antibiotic usage thus suggesting that B. breve plays a signature role in maintaining gut micro biota homeostasis. Commercial probiotics, containing B. breve, are sold in attempts to remedy the drastic decline in species population numbers by supplementing colonization. One study performed in the Netherlands, found that B. breve M16 V may be beneficial in the treatment of chronic asthma by inducing regulatory T cell responses in lung airways of chronically asthmatic mice. Pathology Bifidobacterium breve has not been recorded as the cause of human disease and has been in commercial use as a probiotic since 1976. B. breve is important within the human gut for its ability to metabolize diverse carbohydrates, breaking them down into less complex compounds digestible by the human host. Numerous controlled tests have been conducted prior to marketing in order to ensure the safety of commercial use (Fijan S.2014).

2.5.2 *Bifidobacterium bifidum*

B. bifidum is a bacterial species of the *Bifidobacterium* genus. *B. bifidum* is one of the most common probiotic bacteria that can be found in the body of mammals, including humans. Structure since *B. bifidum* is Gram –positive bacterium that is not motile, anaerobic and not spore-forming. The bacterium is rod-shaped and can be found living in clusters, pairs, or even independently. The majority of the population of B. bifidum is found in the vagina. The use of B. bifidum in probiotic applications may reduce the chances of acute diarrhea and the risk of E coli infections, and contributes to the maintenance of vaginal homeostasis. The health the manipulation of the gut flora is complex and may cause bacteria-host interactions although probiotics in general, are considered safe, there are concerns about their use in certain cases. Some people, such as those with compromised immune system, short bowel syndrome, central venous catheters, heart valve disease, and premature infants, may be at higher risk for adverse events. Rarely, consumption of probiotics may cause bacteremia and sepsis potentially fatal infections in children with lowered immune systems or who are already critically ill. *B. bifidum* is not commonly found in breast milk. Breast feeding is not one way to transmit the bacteria from mother to child. B.

bifidum is found in the vagina; some studies show that vaginal births transmit more *B.bifidum* from mother to child than caesarean births. Transmission of *B. bifidum* allows a child to begin production of micro flora which helps to colonize the child's intestines after birth.

2.5.3 *Bifidobacterium longum*

Bifidobacterium longum gram –positive, negative, rod-shaped bacterium present in the human gastrointestinal tract and one of the 32 species that belong to the genus Bifidobacterium It is a microaerotolerant anaerobe and considered to be one of the earliest colonizers of the gastrointestinal tract of infants. When grown on general anaerobic medium, B. longum forms white, glossy colonies with a convex shape. While B. longum is not significantly present in the adult gastrointestinal tract, it is considered part of the gut flora and its production lactic acid is believed to prevent growth of pathogenic organisms. B. longum is non-pathogenic and is often added to food products. Classification in 2002, three previously distinct species of Bifidobacterium, *B. infantis, B. longum*, and B. suis, were unified into a single species named B. longum with the biotypes infantis, longum, and suis, respectively. This occurred as the three species had extensive DNA similarity including a 16s rRNA gene sequence similarity greater than 97% In addition, the three original species were phenotypically difficult to distinguish due to different carbohydrate fermentation patterns among strains of the same species. As probiotic activity varies among strains of *B. longum*, interest exists in the exact classification of new strains, although this is made difficult by the high gene similarity between the three biotypes. Currently, strain identification is done through polymerase chain reaction (PCR) on the subtly different 16s rRNA gene sequences.

2.5.3.1 Environment

B. longum colonizes the human gastrointestinal tract, where it, along with other Bifidobacterium species, represents up to 90% of the bacteria of an infant's gastrointestinal tract. This number gradually drops to 3% in an adult's gastrointestinal tract as other enteric bacteria such as *Bactericides* and Eubacteria begin to dominate. Some strains of B. longum were found to have high tolerance for gastric acid and bile suggesting that these strains would be able to survive the gastrointestinal tract to colonize the lower small and large intestines. The persistence

of B. longum in the gut is attributed to the glycoprotein binding fimbriae structures and bacterial polysaccharides, the latter of which possess strong electrostatic charges that aid in the adhesion of B. longum to intestinal endothelial this adhesion is also enhanced by the fatty acids in the lipoteichoic of the B. longum cell wall (Sierra S, Lara-Villoslada F, Sempere L, Olivares M, Boza J,et al ,2010).

2.5.3.2 *Bifidobacterium* Genome

Bifidobacterium may exert strong influence in not only colonic health but also in the establishment of the gut environment and shaping of other microbiota successors and the taxonomy of Bifidobacterium has been based on biochemical tests and has progressed rapidly due to the development of modern microbial population genetics, ecology and genomics Members of the Bifidobacterium genus were first identified from the human gastrointestinal tract and were then found to be widely distributed across various ecological niches. Although the genetic diversity of Bifidobacterium has been determined based on several marker genes or a few genomes, the global diversity and evolution scenario for the entire genus remain unresolved. (Olivares M, Diaz-Ropero MA, Gomez N, Lara-Villoslada F, Sierra S, Maldonado JA, et al.2006).

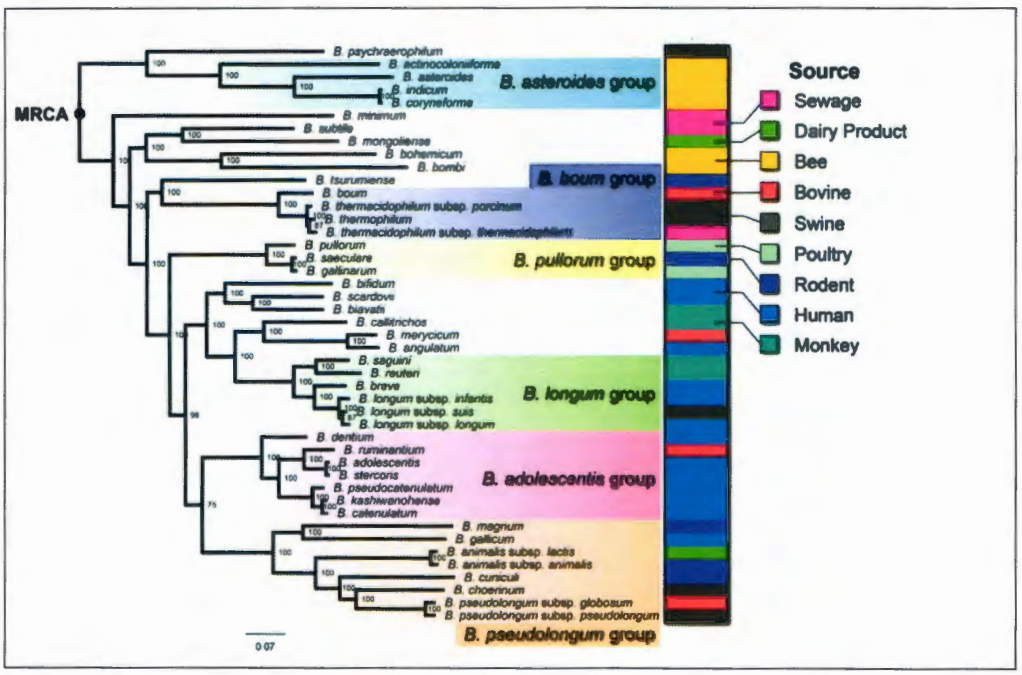

Figure 2.2 Bacteria genome.

Source: Olivares M, et al. (2006)

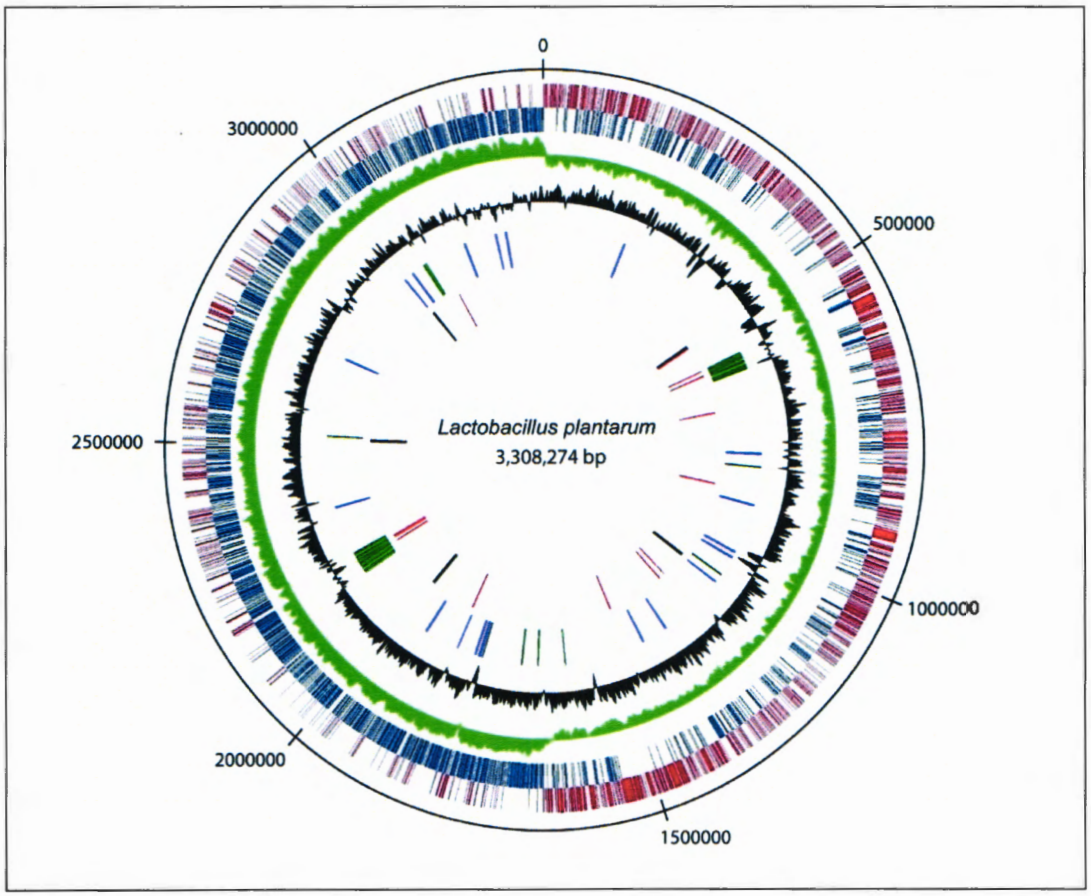

Figure2.3 Lactobacillus genome.

Source: Kleerebezem M, et al. (2003)

Maternal factor and postnatal factors

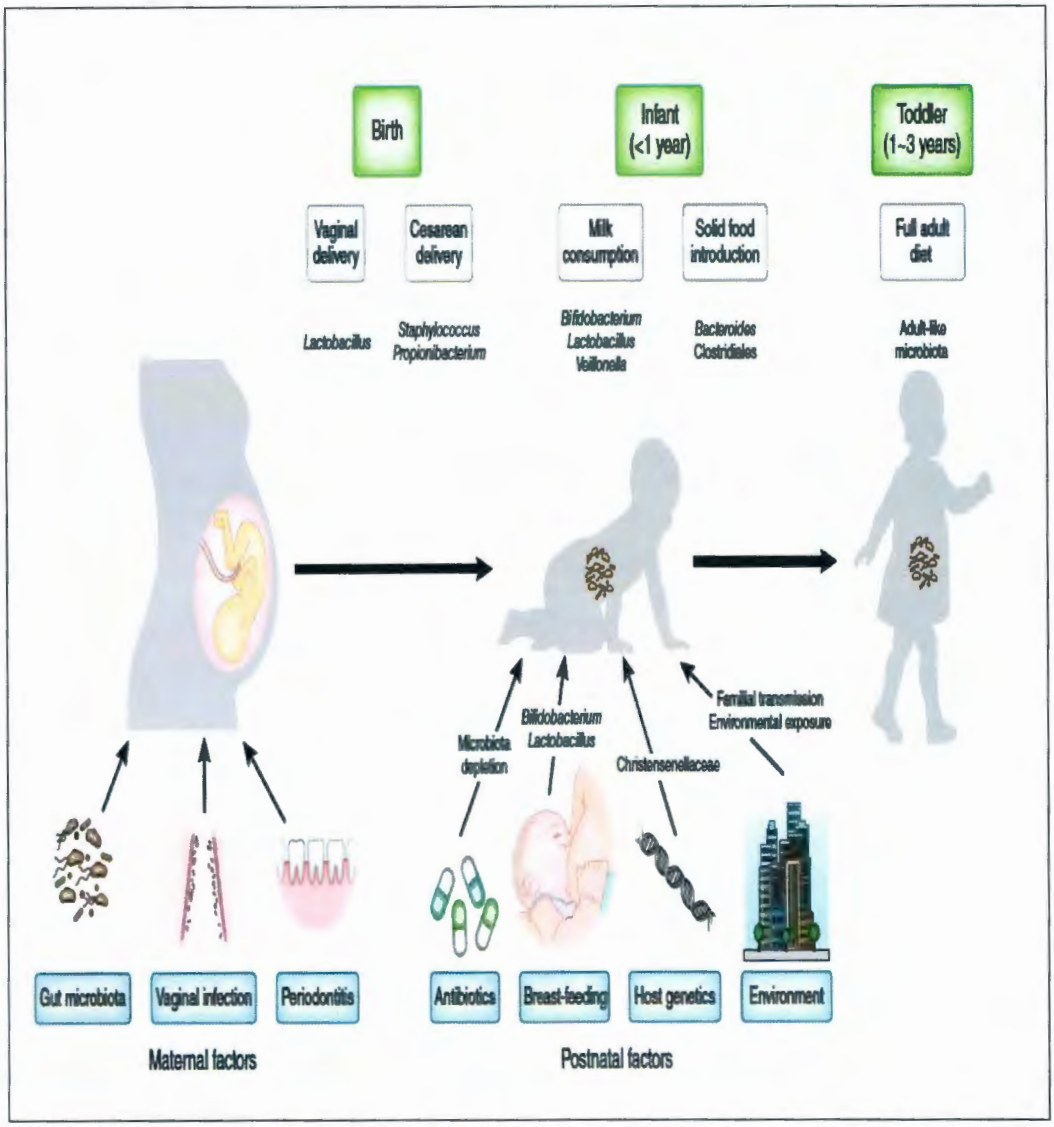

Figure 2.4 The maternal and postnatal factors

Source: Tamburini S, et al. (2016)

2.5.4 *Bifidobacterium* Transmission

The transmission of microbial species to the infant gastrointestinal tract is more difficult to measure but there is elements influence as the placenta is near sterile and likely contributes little to the gut micro biome in the first days of life, also the mother's vaginal canal during birth is the first major source of inoculum. Cesarean section birth limits exposure to possible inoculation both via maternal stool during birth and via vaginal contact. Genomically identical *Bifidobacterium* strains have been

isolated from fecal samples of mother and child combinations, as well as the corresponding human milk samples, indicative of a vertical transmission route from maternal gastrointestinal tract (GIT) to (breastfed) infants. This has given rise to the hypothesis that microbial colonization of the infant depends on the mother's fecal/vaginal microbiota as well as on (providing) breast milk. Bacteria may reach the mammary gland of the mother and may be transmitted directly to breastfed infants, although, inversely, *Bifidobacterium* may be introduced into human milk from the infant's oral cavity during suckling .Also The mother's intestinal microbiota is a likely source of some *Bifidobacterium* for the infant, both during and after the birthing process, and several studies have proven strain congruence among isolates from mother's and infant's feces. The skin of mothers and other caretakers may also be a vector for the early transfer of intestinal microbes (Nami Y, Abdullah N, Haghshenas B, Radiah D, et al., 2014)

2.6 *Lactobacillus*

Is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, nonspore-forming bacteria they are a major part of the lactic acid bacteria group (i.e. they convert sugars to lactic acid). In humans, they constitute a significant component of the microbiota at a number of body sites, such as the digestive, urinary system, and genital system.. In women, Lactobacillus species are normally a major part of the virginal microbiota. Lactobacillus forms biofilms in the vaginal and gut microbiota allowing them to persist during harsh environmental conditions and maintain ample populations. Lactobacillus exhibits a mutualistic relationship with the human body as it protects the host against potential invasions by pathogens, and in turn, the host provides a source of nutrients. Lactobacillus is the most common probiotic found in food such as yogurt, and it is diverse in its application to maintain human well-being as it can help treat diarrhea, vaginal infections and skin disorders such as eczema. (https://en.wikipedia.org/wiki/Lactobacillus).

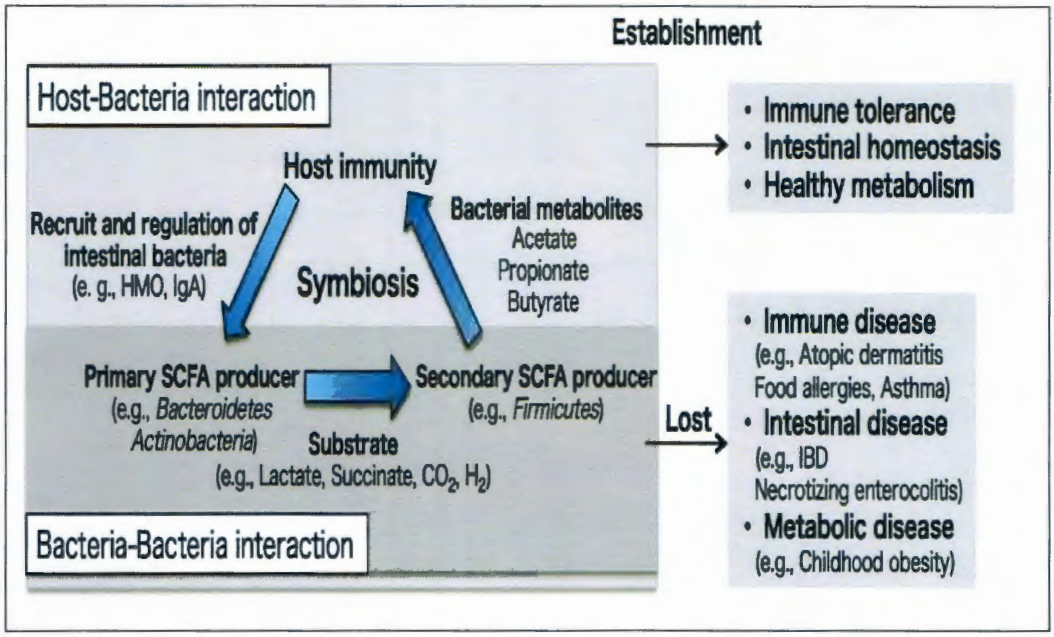

Figure 2.5 Infant's gut microbiota and the host immune system interaction. The resulting gut microbial community and metabolites contributes to the development of the host immune System, intestinal homeostasis and host metabolism.

Source: Tanaka M, et al. (2017)

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Study Design

The work plan of this study is divided into five steps as followed:

Step	Work	Anticipated output
1.	Collection of fecal sample	One sample are collected.
2.	DNA extraction and purification	Extracted DNA with good quality and quantity are obtained.
3.	PCR amplification of 16S rRNA and agarose gel electrophoresis	The specific targeted DNA can be amplified from genomic DNA sample.
4.	Discussion and conclusion	Understand the micro biota of healthy infant feces.
5	Thesis writing and manuscript preparation	Manuscript can be submitted to an appropriate international index journal.

3.2 Ethic permission

One infants, who are born by vagina delivery and have ages from one month to one year, are invited to be volunteer. Ethics statement were approved by the human ethics committee of Ubon Ratchathani University (License approval number: UBU- REC-14/2561). The infant's parent were informed to understood of all research objectives and detailed procedure. And they did agreed and accept all given information, they were invited to sign an informed consent form before participated in the study.

3.3 Fecal samples collection

The inclusion criteria for fecal sample collection from infant includes: 1) infant who are born by vaginal delivery in Ubon Ratchathani province, and has normal body mass index (BMI) ranging from 18.5 to 22.9. The exclusion criteria includes infant was not diagnosed as any recognized diseases or any abnormal conditions. The fecal samples were stored in anaerobic jars with a gas pack (AnaeroPack-Anaerobe, Mitsubishi, Japan) and transported to microbiology laboratory of the College of medicine and public health, Ubon Ratchathani University. The sample was extracted immediately after samples was arrived at the laboratory.

3.4 Genomic DNA extraction

Total DNA is extracted from 200 to 400 mg of isolate DNA from bacteria sample derived from each infant by using Innu PREP Stool DNA Kit according to manufacturer's specifications (Analytikjena Biometra, Jena, and Germany). The quantity and quality of DNA is measured using Nano Drop™ 1000 Spectrophotometer (Thermo Nano drop, MA USA). The total bacterial DNA were stored at -20 °C until further use.

3.5 Oligonucleotide primers for PCR amplification

Oligonucleotide primers used in this study are listed as shown in table 3.1.

Table 3.1 Specific primers based on 16S rDNA sequences

Oligonucleotides	Sequence (5'-3')	Reference
27F	AGAGTTTGATCCTGGCTCAG	S-D-Bact-0008-a-S-20
1492R	GGTTACCTTGTTACGACTT	S-*-Univ-1492R-b-A-21
BiBRE-1	CCGGATGCTCCATCACAC	Matsuki et al.,1998
BiBRE-2	ACAAAGTGCCTTGCTCCCT	Matsuki et al.,1998
BiBIF-1	CCACATGATCGCATGTGATTG	Matsuki et al.,1998
BiBIL-2	CCGAAGGCTTGCTCCCAAA	Matsuki etal.,1998

Table 3.1 Specific primers based on 16S rDNA sequences (continued)

Oligonucleotides	Sequence (5'-3')	Reference
InfU5	CCATCTCTGGGATCGTCGG	Roy et al.,1996
InfU6	TATCGGGGAGCAAGCGTGA	Roy et al.,1996
BIL-1	GTTCCCGACGGTCGTAGAG	Wang et al.,1996
BIL-12	GTGAGTTCCCGGCATAATCC	Wang et al.,1996

3.6 PCR component and condition

Taq polymerase-based amplification was used in this study. PCR was done in 50 µl of PCR mixture, containing 50 mM KCl, 75 mM Tris-HCl (pH 9.0), 20 mM (NH4)2SO2, 1.5 mM MgCl2, 200 µM of each dNTP, 0.2 µM of each specific primer (table) and 1 unit of Taq DNA polymerase. The PCR condition used for amplification of 16S rRNA gene representative of the bacterial is shown in Table 3.2.

Table 3.2 PCR condition used for amplification of 16srDNA gene

Target species	Primers		Amplification condition	Times (sec or min)	Cycles no.	Amplicon sized (bp)
	F	R				
All isolates	27F	1492R	Pre-denaturation (94°C)	4 min	1	1,516
			Denaturation (94°C)	1 min	30	
			Annealing (48°C)	30 sec		
			Extension (72°C)	2 min		
			Final extension (72°C)	10 min	1	

Table 3.2 PCR condition used for amplification of 16srDNA gene (continued)

Target species	Primers		Amplification condition	Times (sec or min)	Cycles no.	Amplicon sized (bp)
	F	R				
B.infantis	InfU5	InfU6	Pre-denaturation (94°C)	4 min	1	565
			Denaturation (94°C)	1 min	30	
			Annealing (55°C)	30 sec		
			Extension (72°C)	2 min		
			Final extension (72°C)	10 min	1	
B.bifidum	BiBIF-1	BiBIF-2	Pre-denaturation (94°C)	4 min	1	278
			Denaturation (94°C)	1 min	30	
			Annealing (50°C)	30 sec		
			Extension (72°C)	2 min		
			Final extension (72°C)	10 min	1	
B.breve	BiBRE-1	BiBRE-2	Pre-denaturation (94°C)	4 min	1	288
			Denaturation (94°C)	1 min	30	
			Annealing (55°C)	30 sec		
			Extension (72°C)	2 min		
			Final extension (72°C)	10 min	1	

Table 3.2 PCR condition used for amplification of 16srDNA gene (continued)

Target species	Primers		Amplification condition	Times (sec or min)	Cycles no.	Amplicon sized (bp)
	F	R				
B.longum	BIL-1	BIL-12	Pre-denaturation (94°C)	4 min	1	153
			Denaturation (94°C)	1 min	30	
			Annealing (55°C)	30 sec		
			Extension (72°C)	2 min		
			Final extension (72°C)	10 min	1	

3.7 Gel electrophoresis

The molecular size of DNA was determined by gel electrophoresis as previously described by Sambr (Sambrook, Russel, 2001). Briefly, the DNA was mixed with the 5X gel-loading buffer in a ratio (1:2). The mixture was then loaded into the wells covered by electrophoresis buffers. The electrophoresis was carried through appropriate voltage (V) until the bromophenol blue have migrated the appropriate distance through the gel. The agarose gel was stained with ethidium bromide and detained with water. Finally, the gel was visualized under UV transillumination. The molecular size of DNA was determined by comparing its bands with standard size DNA.

3.8 DNA Analysis

All analyses was performed uses the IBM SPSS Statistics, version 22.0, statistical software package (IBM Crop, Armonk, NY, USA).Intergroup differences in the number of species are analysis using unpaired t-tests. Spearman's correlation coefficient is used to determine the relationships between bacterial groups for individuals under the detection. For all statements, P-values of 0.05 is consider as statistically significant.

3.9 Site for conducting the study

All experiments in this study were performed at Department of Microbiology, College of Medicine and Public Health, Ubon Ratchathani University, Thailand.

CHAPTER 4
RESULTS

4.1 Isolation of lactic acid bacteria from human feces

One feces sample was collected from volunteer. The feces sample was derived from male infant who was born by cesarean birth and had 2 months old of age. The sample as designated as JB sample. Feces sample was used to isolate LAB. Fifty single colonies with clear zone around their colonies were randomly selected from MRS agar medium containing calcium carbonate at 37 °C under anaerobic conditions. Gram staining revealed that all bacterial isolates were gram-positive with a Y-like and bacilli shape as indicated by arrow in Figure 4.1. All isolates were catalase negative. These preliminary results indicated that all these fifty bacterial isolates could be categorized as LAB and possibility as Bifidobacterium species. Thus these bacterial isolates were used for further steps, including DNA extraction and genus and species identification.

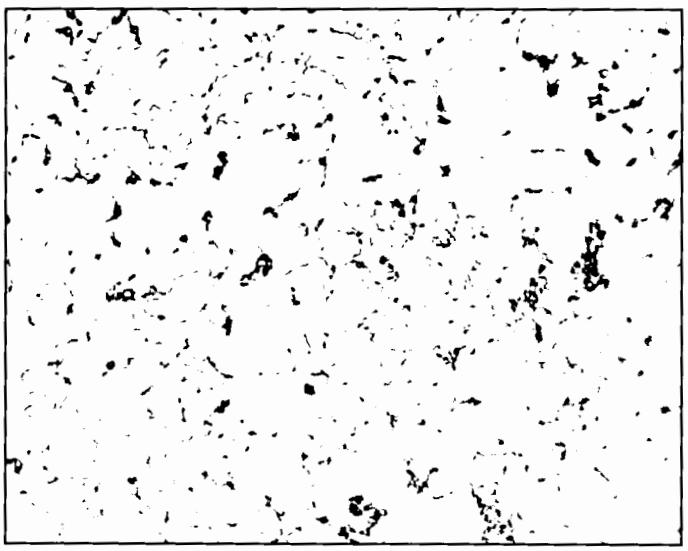

Figure 4.1 Gram staining of JB1, one of fifty bacterial isolates. Arrows indicate Y-like and bacilli shape. N: Negative control. (* indicates the optimal temperature)

4.2 Extraction of Genomic DNA from isolated bacteria

All fifty JB isolates were used for genomic DNA extraction. The quality and quantity of genomic DNA derived from some isolates (JB1 to JB 18) are shown in Table 4.1 . The result demonstrated that genomic DNA extracted from 18 samples showed good quantity and quality that could be appropriate for PCR amplification. Thus, these samples were selected for identification of genus and species by PCR technique and using species-specific primer.

Table 4.1 Quantity and quality of genomic DNA isolated from JB isolates

Isolates	$OD_{260/280}$
JB01	2.25
JB02	2.65
JB03	2.36
JB04	2.58
JB05	2.04
JB06	2.41
JB07	2.48
JB08	2.84
JB09	2.15
JB10	1.70
JB11	2.45
JB12	2.66
JB13	2.26
JB14	2.85
JB15	2.20
JB16	2.26
JB17	3.55
JB18	2.31

4.3 Molecular identification of *Bifidobacterium* species

Firstly, genomic DNA samples of strain JB1, JB2, JB3, JB16, JB18, JB20, JB25, and JB27 were used to amplify the specific 16S rRNA gene which has the length of approximately 1,500 base pair (bp.). The primer pair of InfU5 and InfL6 (Table3.1) and PCR condition (Table3.2) as shown in section of materials and methods were used for amplification. The amplified product was verified by 1% agarose gel electrophoresis. As shown in Figure 4.2, it was found that the amplified product of 16S rRNA gene with approximately of 1,516 bp. could be amplified from the genomic DNA sample of bacterial isolates JB1, JB2 JB3 JB16 JB18 JB20 JB25 JB27 JB31. Thus the genomic DNA was appropriate for species identification by PCR using the specific primers.

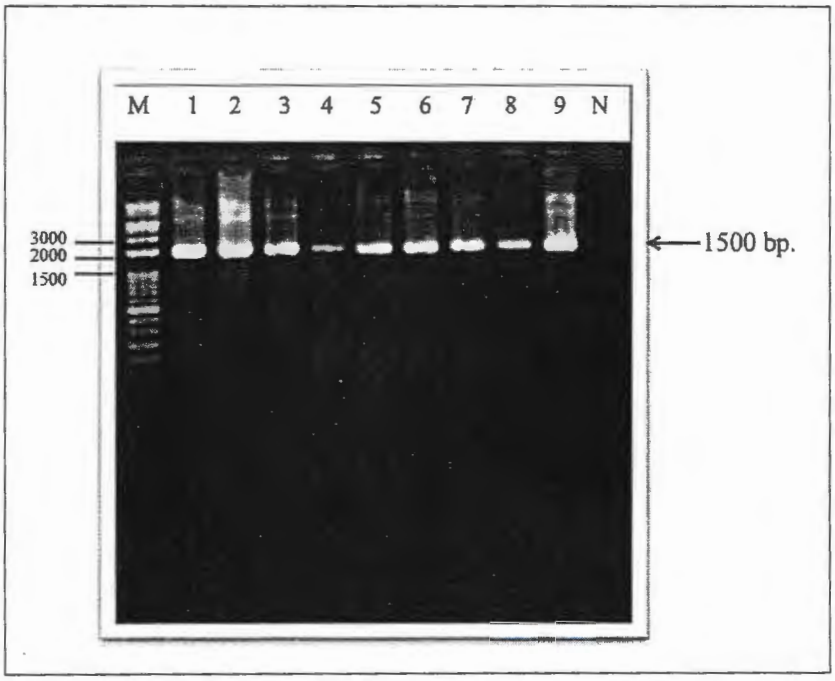

Figure 4.2 Ethidium bromide staining gel of amplified products of 16S rDNA of JB isolates.

Lane M = 1 Kb plus DNA ladder. Lane 1 = amplified product of 16S rDNA gene from Lactobacillus rhamnosus NH3. Lane 2 to 9 = amplified product of 16S rDNA gene from JB1 JB2 JB3 JB16 JB18 JB20 JB25 JB27. Lane N = negative control. Arrow indicates the size of the amplified product in base pair.

4.4 Species specific identification of JB isolates

Based on the result of Gram staining, it was demonstrated that the bacterial isolates could probably be a bacterial group of Bifidobacterium species, as this bacterium has cell morphology of Y-like shape. For these reasons, the specific primers for identification of Bifidobacterium species were used.

Firstly, identification of Bifidobacterium infantis was performed by using the primer pair of InfU5 and InfL6 (Table3.1) and PCR condition (Table3.2). In Figure 4.2 shows the amplification of species-specific 16S rDNA gene for identification of Bifidobacterium infantis from isolates JB1, JB2, JB3, JB4, JB5, JB6, JB7, JB16 and JB18. The result demonstrated that the specific band with the size of 565 bp. was amplified from the genomic DNA sample, indicating that these isolated could be identified as B. infantis .However, some specific bands were still presented in the mixture of amplified PCR product. Thus, the PCR conditions are required for optimization.

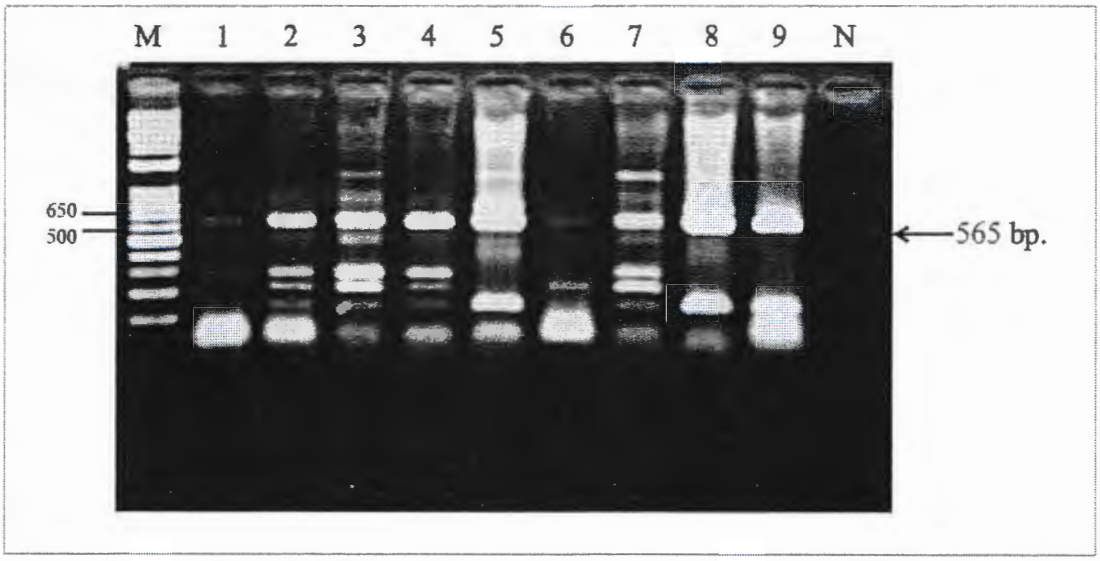

Figure 4.3 Ethidium bromide staining gel of amplified products of 16S rDNA of JB isolates with specific primer InfU5 and InfL6.

Lane M = 1 Kb plus DNA ladder. Lane 1 to 9 = amplified product of 16S rDNA gene from JB1, JB2, JB3, JB4, JB5, JB6, JB7, JB16 and JB18. Lane N = negative control. Arrow indicates the size of the amplified product in base pair.

In Figure 4.3 shows the amplification of 16S rDNA gene for identification of *Bifidobacterium bifidum* from isolates JB1, JB2, JB3, JB4, JB5, JB6, JB7, JB16 and

In Figure 4.3 shows the amplification of 16S rDNA gene for identification of *Bifidobacterium bifidum* from isolates JB1, JB2, JB3, JB4, JB5, JB6, JB7, JB16 and JB18. The PCR was performed by using the primer pair of BiBIF-1 and BiBIF-2. (Table3.1) and PCR condition (Table3.2). The result demonstrated that the specific thin band with the size of 278 bp. was not amplified from all genomic DNA samples, indicating that these isolated could not be classified as *Bifidobacterium bifidum*.

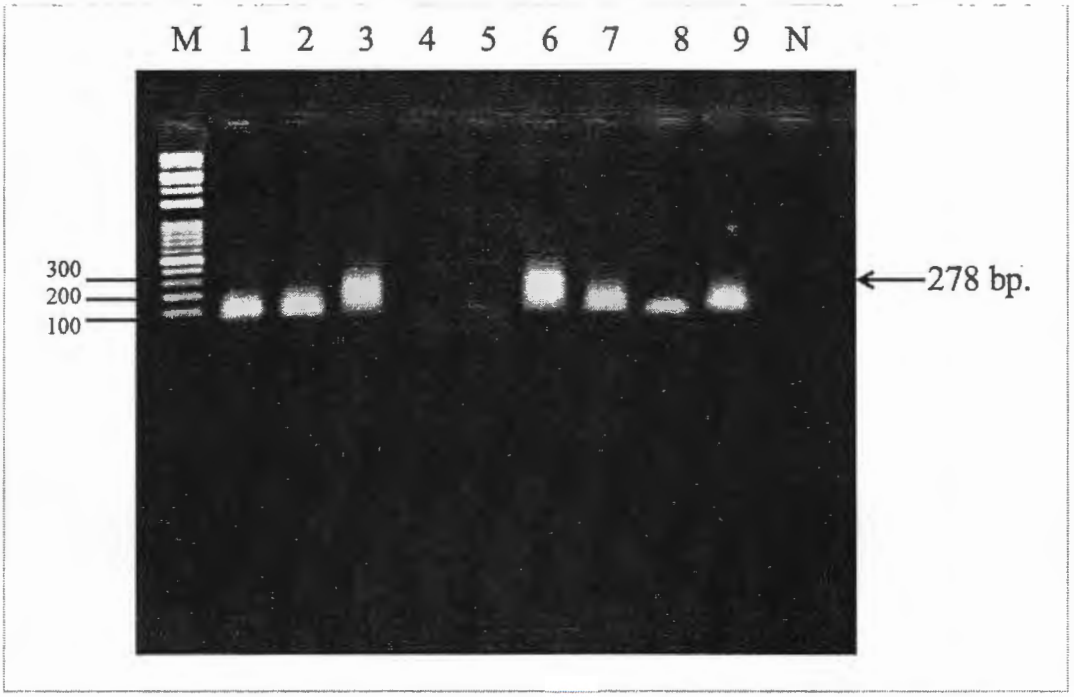

Figure 4.4 Ethidium bromide staining gel of amplified products of 16S rDNA of JB isolates with specific primer BiBIF-1 and BiBIF-2.

Lane M = 1 Kb plus DNA ladder. Lane 1 to 9 = amplified product of 16S rDNA gene from JB1, JB2, JB3, JB4, JB5, JB6, JB7, JB16 and JB18. Lane N = negative control. Arrow indicates the expected size of the amplified product in base pair.

In Figure 4.4 shows the amplification of specific 16S rDNA gene for identification of *Bifidobacterium longum* from isolates JB1, JB2, JB3, JB4, JB5, JB6, JB7, JB16 and JB18. The PCR was performed by using the primer pair of BIL-1 and BIL-2 (Table3.1) and PCR condition (Table3.2). The result demonstrated that the specific band with the size of 153 bp. was not amplified from the genomic DNA, indicating that these isolated could not be identified as *Bifidobacterium longum.*

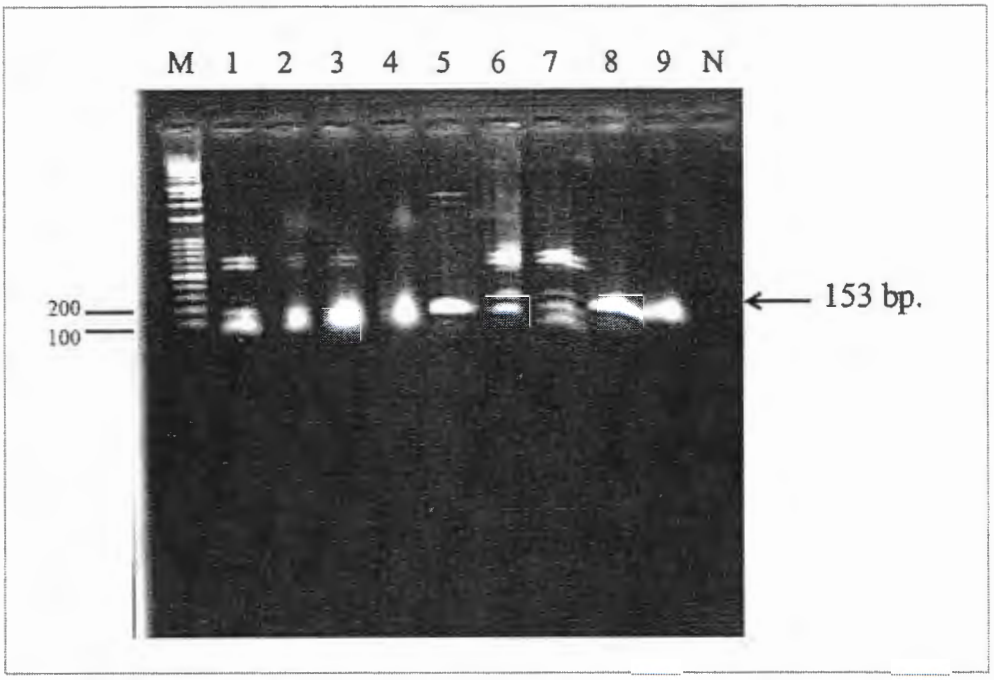

Figure 4.5 Ethidium bromide staining gel of amplified products of 16S rDNA of JB isolates with specific primer BIL-1 and BIL-2.

Lane M = 1 Kb plus DNA ladder. Lane 1 to 9 = amplified product of 16S rDNA gene from JB1, JB2, JB3, JB4, JB5, JB6, JB7, JB16 and JB18. Lane N = negative control. Arrow indicates the size of the amplified product in base pair.

In Figure 4.5 shows the amplification of specific 16S rDNA gene for identification of *Bifidobacterium breve* from isolates JB1, JB2 and JB5. The PCR was performed by using the primer pair of BiBRE-1 and BiBRE-2 (Table3.1) and PCR condition (Table3.2). The result demonstrated that the specific thin band with the size of 288 bp. was amplified from only the genomic DNA sample of isolates JB1, JB2 and JB5, indicating that these isolated could be likely identified as *Bifidobacterium breve*. However, some specific bands were still presented in the mixture of amplified PCR product. Thus, the PCR conditions are required for optimization.

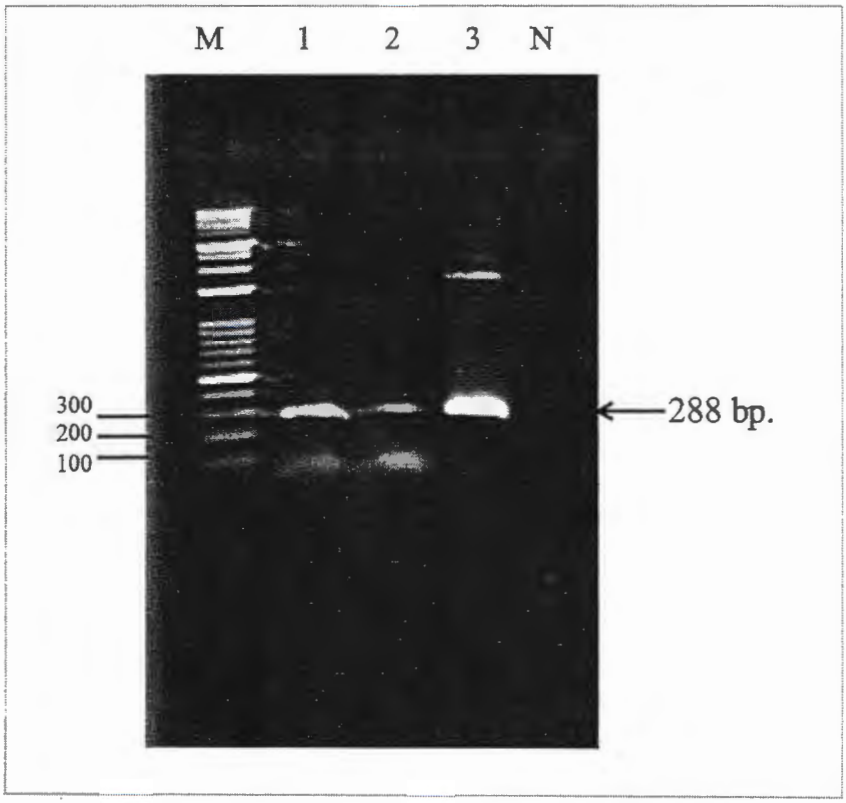

Figure 4.6 Ethidium bromide staining gel of amplified products of 16S rDNA of JB isolates with specific primer BiBRE-1 and BiBRE-2.

Lane M = 1 Kb plus DNA ladder. Lane 1 to 3 = amplified product of 16S rDNA gene from JB1, JB2 and JB5. Lane N = negative control. Arrow indicates the size of the amplified product in base pair.

CHAPTER 5
DISCUSSION AND CONCLUSION

According to the world health organization (WHO), probiotics are defined as live microorganisms that when administered in adequate amounts confer health on the host (Fijan S.2014) Probiotics can be yeast and bacteria (both gram-positive and gram-negative bacteria), but most are members of lactic acid bacteria (LAB), especially in the genus *Lactobacillus* and *Bifidobacterium*. These two bacterial genera are the most abundant LAB group in human gastrointestinal tract. Some species of *Lactobacillus* including *L. casei, L. rhamnosus, L. plantarum, L. reuteri, L. gasseri,* and *L. acidophilus* have been evaluated for their probiotic properties (Belkaid Y, Hand TW, 2014), such as modulation of human immune responses, improvement of the human gut microbiota and intestinal function (Nami Y, Abdullah N, Haghshenas B,et al 2014, Olivares M, Diaz-Ropero MA, Gomez N, Lara-Villoslada F, Sierra S, Maldonado JA, et al.2006). Reduction of the risk of colon cancer. In previous studies, probiotics have been shown to competitively exclude pathogenic intestinal Flora, alter intestinal microflora, reduce carcinogenic secondary bile acids and mutagens, and increase short chain fatty acid production. Probiotics have also been shown to decrease DNA damage at the level of the intestinal mucosa and help maintain an intestinal barrier function (Handler R, Zhang Y, **2018**). Also according to some study said that the fermentation of these prebiotics by beneficial microorganisms under anaerobic conditions they produce Short Chain Fatty-acids (SCFA) like butyrate, acetate, propionate of varying quantities. At the end, it results in a decrease in pH and thereby preventing overgrowth of pathogenic bacteria, which are pH sensitive in nature and it was based broadly on in vitro studies. These short chain fatty acids usually act as a source of carbon for colonocytes and they carry out important metabolic activities like modulation of bioactive food components, vitamin synthesis by intestinal microbiota. Its function shapes the host intestinal anatomy and also gut mucosal immune system (Kumar KS, Sastry N, Polaki H, Mishra Vet alt, 2015),(Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. 2005), and competitive nature against

microbial pathogen (Saavedra JM, Bauman NA, Oung I,et al .1994). For *Bifidobacterium* species, they are able to survive and colonize in the gastrointestinal tract of human (Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, Karlsson PC, et al.2007),to modulate the immune response, to inhibit infection by certain pathogens (Matsuki T, Watanabe K, Tanaka R,2003) and to enhance anticarcinogenic activity (Kullen MJ, Klaenhammer TR ,**2000**) For these supporting reports, the genus *Lactobacillus* and *Bifidobacterium* from human origin, especially from human's gastrointestinal tract are attractive used as probiotic in human.

Lead to this study, we aimed to investigate the two major bacterial genera that includes *Lactobacillus* sp. and *Bifidobacterium* sp. in feces of infants who was born by vagina delivery in Ubon Ratchathani Province. To isolate bacteria from human's gastrointestinal tract, the appropriate sample can be feces, because this sample is easy for sampling and it is not invade human body. Totally, one feces sample was collected from volunteer who was born by cesarean birth. Fifty single colonies with clear zone around their colonies were selected and characterized. Based on Gram staining, it was revealed that all selected bacterial colonies were gram-positive with a Y-like shape, catalase negative, indicated that all these fifty isolates were possibility as *Bifidobacterium* species. It has been suggested that the first step for probiotic characterization was species identification. Some molecular techniques such as 16S ribosomal RNA sequencing have been reported. To date, molecular identification of *Bifidobacterium* species by using species-specific PCR primer have been reported (Matsuki T, Watanabe K, Tanaka R.)For these reasons, the specific primers and PCR condition for identification of *Bifidobacterium* species including *Bifidobacterium infantis, Bifidobacterium bifidum, Bifidobacterium longum* and *Bifidobacterium breve* were used. The result in this study demonstrated that all bacterial isolates were not *Bifidobacterium bifidum* and *Bifidobacterium longum*, due to the specific band were not clearly detected from genomic DNA samples. For *Bifidobacterium breve*, the bacterial isolates JB1, JB2, and JB5 showed the specific band of 288 bp., indicated that this isolate was belonged *B. breve*. However, the isolate JB1, JB2 and JB5 were also identified as *B. infantis,* because the specific band of *B. infantis* with size of 565 bp. was amplified by PCR. To solve this unclear result, the other molecular methods such as complete 16S rRNA sequencing is needed to performed. For identification of

Bifidobacterium infantis, it was demonstrated that all genomic DNA showed non-specific bands in the mixture of amplified PCR product. Thus, the PCR conditions are required for optimization.

Although the result in this study could not lead to the identification of *Bifidobacterium* species, but the knowledge and skill on PCR technique is obtained.

REFERENCES

REFERENCES

Belkaid Y, Hand TW. "Role of the microbiota in immunity and inflammation", **Cell**. 121-41; March, 2014.

Chiang SS, Pan TM. "Beneficial effects of Lactobacillus paracasei subsp paracasei NTU 101 and its fermented products", **Appl Microbiol Biotechnol**. 93(3): 903-16; January, 2012.

Davoodabadi A, and et al. "Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria", **Anaerobe**. 34: 53-8; August, 2015.

Duffy LC, and et al. "Effectiveness of Bifidobacterium bifidum in mediating the clinical course of murine rotavirus diarrhea", **Pediatr Res**. 35(6): 690-5; June, 1994.

Eckburg PB, and et al. "Diversity of the human intestinal microbial flora", **Science**. 308(5728):1635-8; January, 2005.

Eckburg PB, and et al. "Diversity of the human intestinal microbial flora", **Science**. 308(5728): 1635-8; October, 2005.

FAO/WHO. "Guidelines for the evaluation of probiotics in food-Joint Food and Agriculture Organization of the United Nation and World Health Organization Working group Meeting Report", **London Ontario, Canada**. May, 2002.

Fijan S. "Microorganisms with claimed probiotic properties: an overview of recent literature". **Int J Environ Res Public Health**. 11(5): 4745-67; October, 2008.

Graf D, and et al. "Contribution of diet to the composition of the human gut microbiota", **Microb Ecol Health Dis**. 26: 26164; May, 2018.

Hendler R, and et al. "Probiotics in the Treatment of Colorectal Cancer", **Medicines**. 5(3): 101; September, 2018.

Hill CJ, and et al. "Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort", **Microbiome**. 5(1): 4; January, 2017.

REFERENCES (CONTINUED)

Jandhyala SM, and et al. "Role of the normal gut microbiota", **World J Gastroenterol**. 21(29): 8787-803; August, 2015.

Jandhyala SM, and et al. "Role of the normal gut microbiota", **World J Gastroenterol**. 21(29): 8787-803; August, 2015.

Jonkers D, Stockbrugger R. "Probiotics and inflammatory bowel disease", **J R Soc Med**. 96(4): 167-71; April, 2003.

Khajuria N, Metgud R. "Role of bacteria in oral carcinogenesis", **Indian J Dent**. 6(1): 37-43; January - March, 2015.

Kook SY, and et al. "Characterization of the fecal microbiota differs between age groups in Koreans", **Intest Res**. 16(2): 246-54; April, 2018.

Kuang YS, and et al. "Composition of gut microbiota in infants in China and global comparison", **Sci Rep**. 9(6): 36666; November, 2016.

Kullen MJ, Klaenhammer TR. "Genetic modification of intestinal lactobacilli and bifidobacteria", **Current issues in molecular biology**. 2(2): 41-50; April, 2000.

Kumar KS, and et al. "Colon cancer prevention through probiotics: an overview", **J Cancer Sci Ther**. 7(2): 081-92; February, 2015.

Lewis ZT, Mills DA. "Differential Establishment of Bifidobacteria in the Breastfed Infant Gut", **Nestle Nutr Inst Workshop Ser.** 88: 149-59; February, 2017.

Matsuki T, and et al. "Genus- and species-specific PCR primers for the detection and identification of bifidobacteria", **Curr Issues Intest Microbiol**. 4(2): 619; December, 2003.

Murphy K, and et al. "The Composition of Human Milk and Infant Faecal Microbiota Over the First Three Months of Life: A Pilot Study", **Sci Rep**. 17(7): 40597; January, 2017.

Nagpal R, and et al. "Evolution of gut Bifidobacterium population in healthy Japanese infants over the first three years of life: a quantitative assessment", **Sci Rep**. 7(1): 10097; 30 August, 2017.

REFERENCES (CONTINUED)

Nami Y, and et al. "Assessment of probiotic potential and anticancer activity of newly isolated vaginal bacterium Lactobacillus plantarum 5BL", **Microbiol Immunol**. 58(9): 492-502; October, 2014.

Odamaki T, and et al. "Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study", **BMC Microbiol**. 25(16): 90; May, 2016.

Olivares M, and et al. "Oral administration of two probiotic strains, Lactobacillus gasseri CECT5714 and Lactobacillus coryniformis CECT5711, enhances the intestinal function of healthy adults", **Int J Food Microbiol.** 107(2): 104-11; May, 2006.

Rafter J, and et al. "Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients", **Am J Clini Nutr**. 85(2): 488-96; Sep, 2007.

Rajilic-Stojanovic M, and et al. "Diversity of the human gastrointestinal tract microbiota revisited", **Environ Microbiol.** 9(9): 2125-36; September, 2017.

Saavedra JM, and et al. "Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus", **Lancet**. 344(8929): 1046-9; February, 1994.

Sierra S, and et al. "Intestinal and immunological effects of daily oral administration of Lactobacillus salivarius CECT5713 to healthy adults", **Anaerobe**. 6(3): 195-200; January, 2009.

Singh J, and et al. "Bifidobacterium longum, a lactic acid-producing intestinal bacterium inhibits colon cancer and modulates the intermediate biomarkers of colon carcinogenesis", **Carcinogenesis**. 18(4): 833-41; April, 1997.

Talarico ST, and et al. "Anaerobic bacteria in the intestinal microbiota of Brazilian children", **Clinics (Sao Paulo)**. 72(3): 154-60; March, 2017.

Thursby E, Juge N. "Introduction to the human gut microbiota", **Biochem J**. 474(11): 1823-36; May, 2017.

Turroni F, and et al. "Diversity of bifidobacteria within the infant gut microbiota", **PLoS One**. 7(5): e36957; May, 2012.

REFERENCES (CONTINUED)

Wall R, and et al. "Role of gut microbiota in early infant development", **Clin Med Pediatr**. 3: 45-54; October, 2013.

Yasui H, Ohwaki M. "Enhancement of immune response in Peyer's patch cells cultured with Bifidobacterium breve", **J Dairy Sci**. 74(4): 1187-95; April, 1991.

Zhang YJ, and et al. "Impacts of gut bacteria on human health and diseases", **Int J Mol Sci.** 16(4):7493-519; April, 2015.

CURRICULUM VITAE

NAME	Jackson Alor Belbak Meyin
DATE OF BIRTH	May 5, 1980
EDUCATION	Upper Nile University Faculty of Medical and Health Science. Bachelor of Public and Environmental Health Science 2004 -2009.
	School of Health –Malakal, South Sudan.
	2017 - Present, student in Biomedical Sciences.
	Faculty of Medicine and Public Health,Ubon
	Ratchathani University, Thailand,
	Senior project "Study of Fecal Microbiota in Healthy Infant "
INSTITUTION ADDRES	Collage of Medicine and Public Health, Ubon
	Ratchathani University 85 Sathonlamark Road,
	Warin Chamrap, Ubon Ratchathani 34190, Thailand
HOME ADDRESS	Block 7, Gudle –Juba , , South Sudan
	E-mail,wunboing7@gmail.com
	Telephone, +66 990286469
	+211921020009