Preliminary Studies of Lactic Acid Bacteria Isolated from Feces of Thai Newborns

Kruawan Chotelersak PhD*, Tanatip Thamacharoensuk PhD**,
Somboon Tanasupawat PhD**, Kwannan Nantavisai PhD*,
Malai Taweechotipatr PhD*, Srisombat Puttikamonkul PhD*

** Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
*** Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand*

Background: *The microbiota of human plays an important role in the health improvement, and found abundant in the gastrointestinal tract. In recent years, probiotics have been increasingly used in prevention of certain intestinal diseases. The most important population to study the microbiome is probably in the healthy newborns.*
Objective: *The preliminary study aimed to isolate and identify the gut microbiota of newborns for the assessment of prevalent Lactic Acid Bacteria (LAB) distribution.*
Material and Method: *Thirty eight Thai newborns, 0-5 days old of both sexes were subjected for fecal samples collection. Isolated bacteria were cultivated on the MRS selective media and further phenotypically characterized by conventional methods including Gram stain, catalase, and lactic acid production. Genotypic identification was completed by 16S rRNA gene sequencing and phylogenetic analysis.*
Results: *Forty five isolates of LAB and non-LAB bacteria were obtained from feces of newborns. The most prevalence LAB found in this population were 45% Enterococcus faecalis, 14% E. faecium, 11% E. hirae, 11% Lactobacillus paracasei, and 2% L. gasseri. Unusually, Gram-negative bacteria including Klebsiella pneumoniae, Enterobacter hormaechei, Escherichia fergusonii, Leclercia adecarboxylata, and Shigella flexneri were isolated among LAB strains on the selective MRS media.*
Conclusion: *The gut microbiota was a great resource of beneficial LAB which was remarkably distributed among this population of Thai newborns. Further study on individual LAB isolates for the effective probiotics development would be essentially investigated for future alternative treatment of gastrointestinal diseases.*

Keywords: *Enterococcus, Lactobacillus, Lactic acid bacteria, 16S rRNA gene, Newborn, Probiotics*

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Human bodies are made of more bacteria than human cells. It is estimated that 10 times more bacteria than human cells[1]. Among mucosal linings of the human body, the gut alone contains trillions of microbes including bacteria and fungi. Gut microbiota is the most dynamic ecosystem mediated by genetics, diet and environment[2]. Predisposed to altered gut microflora is a major cause of serious illness such as necrotizing enterocolitis, and inflammatory bowel disease (IBD) including ulcerative colitis (UC) and Crohn's disease (CD).

Maintenance of intestinal homeostasis and the development of intestinal diseases, such as IBD are all influenced by the interplay between host factors and microbiota[3,4]. This complex interaction between microbe-derived metabolites and the host immune system is essential for immune cells recognition and functions that influencing the outcome. Several interventions, such as modification of the diet, treatment with antibiotics, application of probiotics and fecal microbiota transplantation, have been introduced into the clinic for the treatment of gastrointestinal diseases. The human gastrointestinal microbiota plays an important role in health improvement, especially in patients with gastrointestinal diseases. In recent years, probiotics have been increasingly used in critical care settings for the prevention of certain diseases that are otherwise associated with high mortality[5,6].

The balance of gut microflora could be restored by consuming certain types of probiotic containing foods[4]. Based on a definition given by the Food and Agriculture Organization of the United Nations World Health Organization (FAO/WHO), probiotics are "live microorganisms which when

Correspondence to:
Puttikamonkul S, Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand.
Phone: +66-2-6495000 ext. 4910
E-mail: srisombat@g.swu.ac.th

administered in adequate amounts confer a health benefit on the host"[7]. Therefore, probiotics should be non-pathogenic, stable in acid and bile, able to adhere to and confer health benefit by colonization of human gut mucosa, and retain viability during storage and use. Many species of microorganisms have been used which mainly consist of lactic acid producing bacteria; *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Bifidobacterium*[8,9].

Screening of new probiotics needs to determine their safety and outstanding research is requisite. Accordingly, the novel species should be isolated from the human fecal microflora of healthy volunteers who have not ingested probiotics containing products for atleast 1 month[10]. Recently, human-derived Lactic Acid Bacteria (LAB) and *Bifidobacterium* were isolated from feces of healthy infants exhibiting their antagonistic interaction against various food-borne pathogenic bacteria[11-13]. Therefore, a prerequisite safety of probiotics isolation is human origin. We hypothesized that microbiota colonized in the gut of newborns was a diversity of beneficial LAB. Therefore, the present study was done by isolation of bacteria from fecal samples from Thai newborns on the MRS selective media and further identified by 16S rRNA gene sequencing.

Material and Method

Subjects and cultivation of fecal samples

Thirty eight fecal samples of healthy Thai newborns were obtained from HRH Princess Maha Chakri Sirindhorn Medical Center, Ongkharak, Thailand. All newborns of both sexes were 0-5 days old. Fresh feces samples were taken by sterile cotton swab and inserted into a modified Cary-Blair transport medium (Oxoid, Hampshire, UK). The samples were processed immediately upon receipt. Each individual's parents have been informed and signed the consent. This study was approved by the Research ethics committee of Srinakharinwirot University (SWUEC 37/2551).

The fecal samples were diluted in normal saline solution (NSS) and plated in duplicate by spreading onto de Man-Rogosa-Sharpe (MRS) agar plates (Oxoid, Hampshire, UK), a selective media for lactic acid bacteria isolation. The plates were incubated under anaerobic condition at 37°C for 48-72 hr under anaerobic condition using anaerobic GasPak (Mitsubishi, Japan) in an anaerobic box (Mitsubishi, Japan)[14]. All isolated bacteria which presenting different colony morphologies from each feces sample were collected on new MRS agar plates. After anaerobic incubation, single pure colonies were isolated and sub-cultured for further experimental use.

Screening of Lactic Acid Bacteria (LAB) isolates

Each isolate was screened based on Gram reaction, catalase test, and lactic acid production. To test for catalase activity, bacteria were mixed with 3% H_2O_2 and immediate formation of bubbles indicated the presence of catalase. For lactic acid production, bacteria isolates were cultured on MRS agar containing 0.3% $CaCO_3$ and incubated at 37°C for 48-72 hr under anaerobic condition[15]. Consequently, LAB isolates were selected by the presence of a clear zone around the colonies. All characterized isolates were maintained as frozen cultures in MRS broth with 20% glycerol at -80°C for further studies.

Amplification of 16S rRNA gene and sequencing

DNA was extracted from cell pellets using a Wizard Genomic DNA purification kit (Promega AG, Switzerland) according to the manufacturer's instructions and strain were typed to species level using 16S rRNA gene sequencing as described[15]. Briefly, the template DNA for sequencing of the 16S rRNA gene was amplified using universal primers: 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). For DNA sequencing, two sets of primers were used for full length sequences alignment including primers 27F, 518F (5'-CCAGCAGCCGCGGTAATACG-3'), 800R (5'-TACCAGGGTATCTAATCC-3') and 1492R, and were sequenced by Macrogen, Korea.

Polymerase chain reactions (PCR) were performed in a total volume of 50 µl containing 2 µl of genomic DNA, 10 pmol of each primer, 2.5 U Taq DNA polymerase (Applied Biosystems, Foster City, CA, USA), 2 mM dNTP, 1x reaction buffer and 25 mM $MgCl_2$. The reaction was thermally cycled as follows: an initial denaturation at 94°C for 3 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min and a final extension of 72°C for 3 min with a GeneAmp PCR system 2700 (Applied Biosystems, Foster City, CA, USA).

The similarity of 16S rRNA gene sequences were determined using BLAST software compared to EzTaxon-e database[16]. Multiple alignments of sequences were performed by CLUSTAL X version 2.0.11. The alignment was modified by eliminate gaps and ambiguous nucleotides prior to the construct a phylogenetic tree. The phylogenetic tree was constructed with neighbor-joining in the MEGA 6

software[17]. The confidence values of individual branches in the phylogenetic tree were determined with 1,000 replications.

Results

Isolation of lactic acid bacteria isolates

Initial phenotypic characterization of bacterial isolates collected from 38 fecal samples obtained from Thai newborns including Gram stain, catalase activity and lactic acid production. A total of 45 isolates were facultative anaerobes including 37 isolates of Gram-positive bacteria and 8 isolates of Gram-negative bacteria. Bacterial characteristics were summarized in Table 1. Cell morphology of isolates varied from long and slender rods, straight rods to bent rods, and cocci. The most frequently found colonies varied from small to medium colonies (1-2 mm) with white, circular, smooth and convex colonial morphologies.

Genotyping of selected LAB isolates based on 16S rRNA gene sequences

A list of 45 isolates obtained from 38 Thai newborns was identified with published 16S rRNA gene sequences. The amplified products of complete 16S rRNA gene with expected length of 1,200-1,500 bp after quality trimming were aligned for genotypic identification using BLAST, resulting the distribution of 45% *Enterococcus faecalis* (20 isolates), 14% *Enterococcus faecium* (6 isolates), 11% *Enterococcus hirae* (5 isolates), 11% *Lactobacillus paracasei* (5 isolates), 2% *Lactobacillus gasseri* (1 isolate), 9% *Klebsiella pneumoniae* (4 isolates), 2% *Enterobacter hormaechei* (1 isolate), 2% *Escherichia fergusonii* (1 isolate), 2% *Leclercia adecarboxylata* (1 isolate) and 2% *Shigella flexneri* (1 isolate) (Fig. 1). Estimates of evolutionary divergence between 16S rRNA gene sequences were conducted using the Kimura 2-parameter model in MEGA[18]. The results showed that each species were different less than 2% divergence concurred with the threshold to constitute species (range 0.03-0.28%) (Table 2). The 16S rRNA gene sequences of all 45 isolates show high homology similarity to the reference species in GenBank database (98-100%) (Table 3).

Phylogenetic tree analysis

Phylogenetic tree was constructed by a neighbor-joining method with 16S rRNA gene sequences of present isolates of LAB and other isolates were shown their taxonomic positioning in the Fig. 2. Isolates were divided into 3 distinct clades including one clade of Gram-positive, cocci, *E. faecalis*

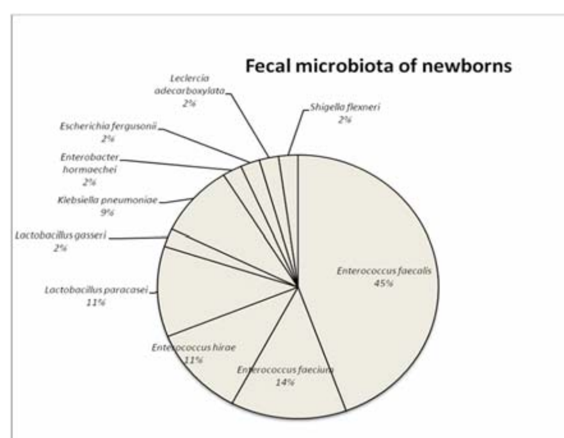

Fig. 1 Distribution of isolated bacterial species in the fecal microbiota of newborns (n = 45).

Table 1. Phenotypic characterization and prevalence of isolated bacterial species in the newborns fecal samples (n = 45)

Bacterial species	Gram stain	Catalase	Lactic acid production	Distribution of species (n = 45)
Enterococcus faecalis	(+)	(-)	+	20 (45%)
Enterococcus faecium	(+)	(-)	+	6 (14%)
Enterococcus hirae	(+)	(-)	+	5 (11%)
Lactobacillus paracasei	(+)	(-)	+	5 (11%)
Lactobacillus gasseri	(+)	(-)	+	1 (2%)
Klebsiella pneumoniae	(-)	(+)	-	4 (9%)
Enterobacter hormaechei	(-)	(+)	-	1 (2%)
Escherichia fergusonii	(-)	(+)	-	1 (2%)
Leclercia adecarboxylata	(-)	(+)	-	1 (2%)
Shigella flexneri	(-)	(+)	-	1 (2%)

Table 2. Percentage intraspecific variation for the 16s rRNA gene of isolates species obtained from feces of Thai newborns using Kimura 2-Parameter model

Species	% Divergence			
	Mean	SE	Minimum	Maximum
Enterococcus faecalis	0.03	0.01	0	0.37
Enterococcus faecium	0.11	0.03	0	0.39
Enterococcus hirae	0.07	0.02	0	0.22
Lactobacillus paracasei subsp. *tolerans*	0.11	0.03	0	0.33
Klebsiella pneumoniae subsp. *rhinoscleromatis*	0.28	0.05	0.07	0.52

with closely related species, *E. faecium*, and *E. hirae*. One clade of Gram-positive, rod, *L. paracasei* and *L. gasseri*, and one clade of Gram-negative, rod, *K. pneumoniae*, *E. hormaechei*, *E. fergusonii, L. adecarboxylata, S. flexneri*. For this analysis, *Bacteriodes fragilis*, an obligate anaerobic, Gram-negative, rod-shaped bacterium which is part of the normal flora of the human colon and is generally commensal, was used as outlier species.

Discussion

The prevalence commensally isolates of this study were LAB including 70% of *Enterococcus* and 13% *Lactobacillus* genera. Although the genus *Bifidobacterium* is the most prevalent gut flora in human infants[19], none of isolates obtained from 38 Thai healthy newborns was found. Recent study on the bacteria isolation of feces from Kenyan infants found that a broad diversity of *Bifidobacterium* were isolated from iron limited condition of 6 months old infants (i.e. anemic, low hemoglobin level)[19]. The data suggested that those *Bifidobacterium* utilized iron sequestration mechanism such as siderophore production and iron internalization for competition against enteropathogens. Given that previous finding confirmed the ability of *Lactobacillus* to grow in the presence and absence of iron[20], our data suggested that gut microenvironment was not restricted for colonization of *Lactobacillus*. In addition, genus *Enterococcus* was the most prevalence LAB (70%) found in this Thai newborns population.

Unfortunately, the transport media and enrichment media MRS used in this study may not be able to obtain and cultivate the *Bifidobacterium*. In the future, appropriate media will be considering for some strictly anaerobe such as *Bifidobacterium*. Moreover, the growth requirements of many bacteria are unknown, most of the gastrointestinal bacteria remain uncultivated. To study the microbial diversity, several studies avoiding the cultivation error and utilized molecular-based studies[21,22]. Moreover, it was unexpected that Gram-negative bacteria including *K. pneumoniae*, *E. hormaechei*, *E. fergusonii, L. adecarboxylata,* and *S. flexneri* would be obtained from MRS selective media which was commonly used for LAB isolation. It has been reported that *K. pneumoniae* was isolated from stool samples of healthy subjects and hospitalized patients, whereas *E. fergusonii* was found in the faecal samples of chicken flocks[23,24]. The possibly reason for our finding could be due to the contamination during the mothers and newborns were hospitalized in the hospital. However, all non lactic acid bacteria were not excluded from the experiment and further analysis for species to ensure the accuracy of the identification result.

A highly conserved sequence of 16S rRNA gene was utilized for species identification by BLAST algorithm analysis with published sequences. Above 98% homology similarity was found in all 45 isolates (Table 3) including the minority cluster that belong to pathogenic bacteria rather than beneficial bacteria such as *S. flexneri* and *E. hormaechei*. First inoculum of microbes in infants was acquired from their mothers during their birth. Mother-infant transmission is mediated by mode of birth, and accumulating data suggested that the microbiota of vaginally born infants is more similar to the vaginal and faecal microbiota of their mother, whereas caesarean section influences a microbiota consisting of the mother's skin and the environment[25,26]. Therefore, the finding of Gram-negative bacteria in the minority clade (Fig. 2) is corresponding to newborns that were born via vaginal delivery.

Conclusion

In the present study, LAB (*Enterococcus* and

Table 3. 16S rRNA gene sequences similarity showing species of 45 bacterial strains isolated from feces of Thai newborns

Isolate No.	Nearest relatives	GenBank	Sequence similarity	
		Accession No.	%	Size (bp)
MSMC10-3	*Enterococcus faecium* LMG 11423^T	AJ301830.1	99	1358
MSMC12-3	*Leclercia adecarboxylata* CIP 82.92^T	JN175338.1	99	1375
MSMC14-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	100	1401
MSMC15-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	100	1409
MSMC15-4	*Enterococcus faecalis* DSM 20478^T	LN681572.1	100	1398
MSMC23-4	*Lactobacillus gasseri* ATCC 33323^T	AF519171.1	99	1420
MSMC24-3	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1395
MSMC35-3	*Enterococcus faecalis* DSM 20478^T	LN681572.1	100	1408
MSMC36-7	*Lactobacillus paracasei* subsp. *tolerans* NBRC 15906^T	AB181950.1	99	1414
MSMC36-8	*Lactobacillus paracasei* subsp. *tolerans* NBRC 15906^T	AB181950.1	99	1404
MSMC40-5	*Klebsiella pneumoniae* subsp. *rhinoscleromatis* ATCC 13884^T	Y17657.1	99	1385
MSMC42-2	*Lactobacillus paracasei* subsp. *tolerans* NBRC 15906^T	AB181950.1	99	1429
MSMC43-2	*Lactobacillus paracasei* subsp. *tolerans* NBRC 15906^T	AB181950.1	100	1222
MSMC46-2	*Enterococcus faecium* LMG 11423^T	AJ301830.1	99	1407
MSMC47-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1420
MSMC49-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1397
MSMC49-4	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1414
MSMC49-6	*Enterococcus hirae* DSM 20160^T	Y17302.1	99	1425
MSMC49-8	*Enterococcus hirae* DSM 20160^T	Y17302.1	99	1373
MSMC52-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1419
MSMC56-2	*Shigella flexneri* ATCC 29903^T	X96963.1	99	1348
MSMC57-4	*Enterococcus faecalis* DSM 20478^T	LN681572.1	100	1403
MSMC58-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1411
MSMC61-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	98	1406
MSMC61-4	*Enterobacter hormaechei* CIP 103441^T	AJ508302.1	99	1372
MSMC62-3	*Escherichia fergusonii* ATCC 35469^T	AF530475.1	99	1393
MSMC63-4	*Klebsiella pneumoniae* subsp. *rhinoscleromatis* ATCC 13884^T	Y17657.1	99	1389
MSMC64-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1400
MSMC65-2	*Klebsiella pneumoniae* subsp. *rhinoscleromatis* ATCC 13884^T	Y17657.1	99	1380
MSMC66-2	*Klebsiella pneumoniae* subsp. *rhinoscleromatis* ATCC 13884^T	Y17657.1	99	1386
MSMC69-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1400
MSMC71-3	*Enterococcus faecalis* DSM 20478^T	LN681572.1	100	1401
MSMC71-6	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1416
MSMC75-2	*Enterococcus faecium* LMG 11423^T	AJ301830.1	99	1362
MSMC76-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	100	1410
MSMC77-1	*Enterococcus faecium* LMG 11423^T	AJ301830.1	98	1321
MSMC80-2	*Enterococcus faecium* LMG 11423^T	AJ301830.1	99	1372
MSMC95-5	*Lactobacillus paracasei* subsp. *tolerans* NBRC 15906^T	AB181950.1	99	1409
MSMC99-4	*Enterococcus hirae* DSM 20160^T	Y17302.1	99	1426
MSMC100-1	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1408
MSMC118-2	*Enterococcus hirae* DSM 20160^T	Y17302.1	99	1378
MSMC123-1	*Enterococcus hirae* DSM 20160^T	Y17302.1	99	1403
MSMC174-1	*Enterococcus faecium* LMG 11423^T	AJ301830.1	99	1402
MSMC241-3	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1416
MSMC245-2	*Enterococcus faecalis* DSM 20478^T	LN681572.1	99	1414

Lactobacillus) were a major cultivable gut microbiota of collected feces from a population of Thai newborns. The conventional method of identifying LAB strains relies on phenotypic characterization. The selective MRS media, Gram stain, catalase, and lactic acid production were mainly used for screening.

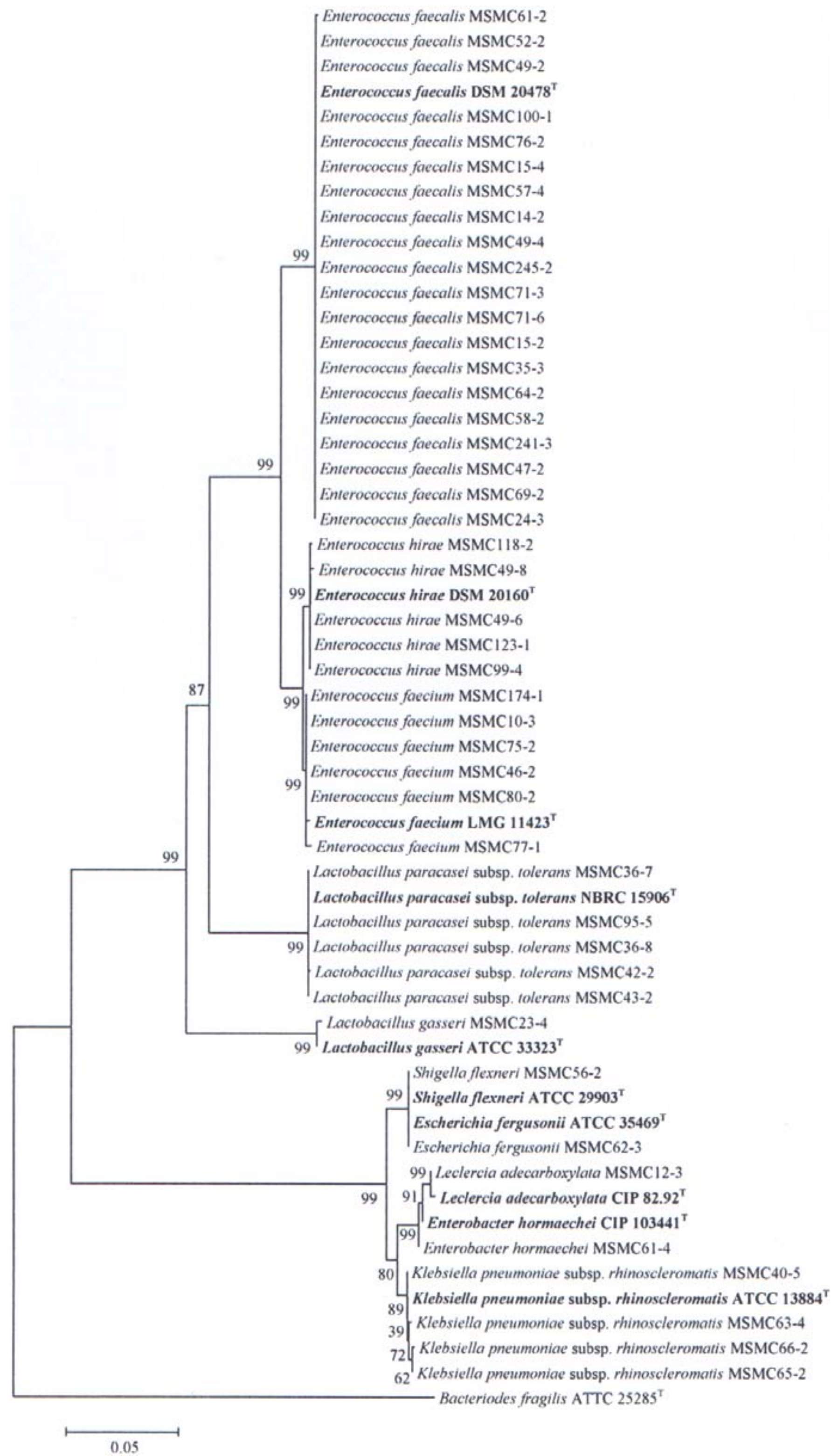

Fig. 2 Neighbour-joining concatenated phylogenetic tree of the 16S rRNA sequences of 45 isolated strains from feces of Thai newborns and GenBank database (bold name). The tree is rooted using *Bacteriodes fragilis* as an out-group.

Accordingly, the conserved 16S rRNA gene was a great molecular tool for bacterial species-subspecies identification. In addition, phylogenetic analysis supported that the prevalent LAB species truly distributed in a population of Thai newborns. Further study on individual LAB isolates for seeking the effective probiotic bacteria will be continuing investigated.

What is already known on this topic?

The colonization pattern of the human gut microbiota begins during birth and influencing by microbes from mothers and environment. Several studies have been done in the infants showing that there was variation between individuals and greater microbial diversity between infants than adults. However, the diversity of gut microbiota has never been investigated in Thai newborns.

What this study adds?

Our preliminary data suggested that *Enterococcus* and *Lactobacillus* genera are the most prevalent LAB colonized in the gut of Thai newborns population. Accordingly, the healthy newborns are the important microbiome resource for new probiotics seeking and development.

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Potential conflicts of interest

None.

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การศึกษาเบื้องต้นของแบคทีเรียกรดแลคติกที่แยกได้จากอุจจาระเด็กทารกไทย

เครือวัลย์ โชติเลอศักดิ์, ธนาทิพย์ ธรรมาเจริญสุข, สมบูรณ์ ธนาศุภวัฒน์, ขวัญนันท์ นันทวิสัย, มาลัย ทวีโชติภัทร์, ศรีสมบัติ พุฒิกมลกุล

ภูมิหลัง: *จุลินทรีย์ประจำถิ่นในร่างกายมนุษย์มีบทบาทสำคัญในการสร้างเสริมสุขภาพ ซึ่งจะพบแบคทีเรียเหล่านี้จำนวนมากในทางเดินอาหาร ในหลายปีที่ผ่านมาได้มีการนำแบคทีเรียที่ดี คือ โพรไบโอติกไปใช้ในการป้องกันการเกิดโรคทางเดินอาหารเกี่ยวกับลำไส้เพิ่มมากขึ้น โดยกลุ่มประชากรที่เหมาะสมต่อการศึกษาเกี่ยวกับจุลินทรีย์เหล่านี้คือเด็กทารกแรกเกิดที่มีสุขภาพดี*

วัตถุประสงค์: *การศึกษาเบื้องต้นเพื่อแยกและจำแนกเชื้อแบคทีเรียประจำถิ่นในทางเดินอาหารของเด็กทารกแรกเกิด และเพื่อประเมินการกระจายตัวของกลุ่มแบคทีเรียกรดแลคติก*

วัสดุและวิธีการ: *เก็บตัวอย่างอุจจาระจากกลุ่มตัวอย่างเด็กทารกแรกเกิดทั้งเพศหญิงและชาย อายุ 0-5 วัน จำนวน 38 คน มาแยกเชื้อแบคทีเรียบนอาหารชนิด MRS แล้วนำเชื้อที่เจริญได้บนอาหารชนิดนี้ไปศึกษาคุณลักษณะฟีโนไทป์เบื้องต้นตามวิธีการดั้งเดิมด้วยการย้อมแกรม การทดสอบคะตะเลส และการสร้างกรดแลคติก หลังจากนั้นจำแนกชนิดของเชื้อแบคทีเรียด้วยวิธีเปรียบเทียบลำดับเบสของจีน 16S rRNA และนำข้อมูลที่ได้มาวิเคราะห์ความสัมพันธ์ของแบคทีเรียที่แยกได้ด้วย phylogenetic analysis*

ผลการศึกษา: *แบคทีเรียที่แยกได้จากอุจจาระเด็กทารกทั้งสิ้น 45 สายพันธุ์ ประกอบด้วย กลุ่มที่สร้างกรดแลคติก (LAB) และกลุ่มที่ไม่สร้างกรดแลคติก สัดส่วนของเชื้อแบคทีเรียกลุ่ม LAB ที่พบในกลุ่มประชากรนี้ คือ Enterococcus faecalis 45%, E. faecium 14%, E. hirae 11%, Lactobacillus paracasei 11%, และ L. gasseri 2% ตามลำดับ นอกจากนี้ยังพบเชื้อแบคทีเรียแกรมลบ Klebsiella pneumoniae, Enterobacter hormaechei, Escherichia fergusonii, Leclercia adecarboxylata, และ Shigella flexneri ซึ่งเจริญบนอาหารเลี้ยงเชื้อ MRS ที่จำเพาะต่อการแยกเชื้อแบคทีเรียกลุ่ม LAB ได้*

สรุป: *จากผลการทดลองที่ได้แสดงให้เห็นว่าทางเดินอาหารของเด็กทารกเป็นถิ่นอาศัยของเชื้อ LAB ชนิดมีประโยชน์ ซึ่งพบกระจายตัวเด่นชัดในกลุ่มประชากรเด็กทารกไทย ดังนั้นการนำเชื้อ LAB ที่แยกได้แต่ละตัวไปทำการศึกษาต่อไป เพื่อค้นหาโพรไบโอติกที่มีประสิทธิภาพ เป็นการศึกษาวิจัยที่สำคัญในอนาคตและมีประโยชน์สูงในการใช้เป็นการรักษาทางเลือกในกลุ่มผู้ป่วยโรคทางเดินอาหารได้*