Antimicrobial Properties of a Potential Probiotic *Lactobacillus* from Thai Newborn Feces

Juntana Chimchang MSc*, Talent Theparee MD*,
Boonyarut Ladda MSc*, Somboon Tanasupawat PhD**,
Benjamas Thanomsub Wongsatayanon PhD*, Malai Taweechotipatr 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: *Probiotics are increasingly used to treat infectious diarrhea and antibiotic-associated diarrhea. Many probiotic bacteria are classified in general such as Lactobacillus and are able to colonize the gastrointestinal tracts of infants.*
Objective: *This study was performed to detect antimicrobial substances and activity in 200 Lactobacillus isolates obtained from healthy Thai newborn feces.*
Material and Method: *Reuterin production was detected by the spot overlay technique and colorimetric assay. Antimicrobial activity was tested by using a well diffusion, agar method.*
Results: *Lactobacillus strain MSMC64-1 produced reuterin and demonstrated potent antimicrobial activity against seven pathogenic indicator strains with very strong inhibitory activities against Salmonella typhi DMST 5784 and methicillin resistant Staphylococcus aureus (MRSA) DMST 20651. There was strong inhibitory activity against methicillin resistant Staphylococcus aureus (MRSA) DMST 20654, Vibrio parahaemolyticus DMST 5665 and Shigella dysenteriae DMST 15111. There was moderate to weak inhibitory activities against Vibrio cholerae DMST 2873 and Helicobacter pylori (H40). The Lactobacillus strain MSMC 64-1 showed resistance to acidic pH (pH 2, 3, 4) and tolerance to 1%, 2%, 3%, and 4% bile concentrations. Sequencing of the 16S ribosomal DNA identified the candidate's strain as Lactobacillus reuteri with 98% sequence homology.*
Conclusion: *The active isolate could potentially be used as a probiotic to prevent and treat enteric infections.*

Keywords: *Probiotics, Antimicrobials, Lactobacillus*

J Med Assoc Thai 2015; 98 (Suppl. 9): S116-S122
Full text. e-Journal: *http://www.jmatonline.com*

Probiotics are defined as "live micro-organisms, which when administered in adequate amounts, confer a health benefit on the host"[1]. Important characteristics for probiotic bacteria include ability to survive the effects of acid and bile in host gastrointestinal tracts and exert desired effects. Most probiotic bacteria are similar to the normal flora in the human gut, especially in breast fed infants, which are known to provide natural protection against many pathogens[2,3].

Probiotics have also shown efficacy in the treatment of infectious diarrhea[4] and antibiotic associated diarrhea[5]. It is believed that probiotics compete with pathogens for adhesion with epithelial cells, metabolic substrates, immune system modulation, and production of antimicrobial compounds[6,7].

Reuterin or 3-hydroxypropionaldehyde (3-HPA) is a pH-neutral, water soluble substance that is produced as an intermediate metabolite during anaerobic fermentation of glycerol[8] by some strains of *Lactobacillus*[9], *Citrobacter*[10], and *Bacillus*[11]. Reuterin has shown good broad-spectrum effects against a range of food-borne pathogens including Gram-positive and Gram-negative bacteria, as well as several fungi and protozoa[8,12-15].

The mechanism of action of reuterin is believed to be due to the inhibition of ribonucleotide reductase (NRD), which is involved in the first step of de novo synthesis of deoxyribonucleotides. Recent reports have also suggested that the mechanism of action involving oxidative stress and ultimately leading to bacterial cell death[13,16,17].

Correspondence to:
Taweechotipatr M, Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, Bangkok 10110, Thailand.
Phone: +66-2-6495341, Fax: +66-2-6495394
E-mail: malai@g.swu.ac.th

The aim of the present study is to isolate and identify novel reuterin-producing *Lactobacillus* strains from infant feces and to determine its antimicrobial properties against selected pathogens for use as potential probiotics. The strains were tested for suitable probiotic characteristics including survival in simulated gastrointestinal conditions using bile salts and acidic environments for potential use as a probiotic.

Material and Method

Sample collection

Fecal samples were collected from 200 healthy newborns at the Department of Obstetrics and Gynecology, HRH Princess Maha Chakri Sirindhorn Medical Center, Nakhon Nayok, Province, Thailand, after informed consent was obtained. All research conducted with human samples were approved by an ethics committee (authorization No. SWUEC 37/2551). Fresh fecal specimens were collected using sterile cotton swabs and transported in Cary-Blair transport medium (Oxoid, Hampshire, England).

Bacterial isolation and culture

For the isolation of *Lactobacillus* spp., each sample was diluted in normal saline solution (NSS) and 10-fold serial dilutions were diluted to 10^{-6}. Each diluted solution was spread-plated onto de Man-Rogosa-Sharpe (MRS) agar plates and incubated at 37°C for 24-48 hour under anaerobic conditions in an anaerobic box (Mitsubishi, Japan). Colonies of different morphologies were isolated on MRS agar plates. After anaerobic incubation, single pure colonies were isolated and sub-cultured for experimental use. Each of the *Lactobacillus* isolates was presumptively identified by Gram reaction and catalase test. Selected isolates of Gram-positive and catalase-negative bacteria were maintained as frozen cultures in MRS broth with 20% glycerol at -80°C.

Pathogenic bacterial strains and growth conditions

Seven bacterial pathogens were used in this study including methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20651, methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20654, *Vibrio cholerae* DMST 2873, *Vibrio parahaemolyticus* DMST 5665, *Salmonella typhi* DMST 5784, and *Shigella dysenteriae* DMST 15111. Pathogens were inoculated on Mueller-Hinton agar (MH: Difco, USA) and incubated under aerobic conditions at 37°C for 24 hour. *Helicobacter pylori* (H40) was grown on brain heart infusion (BHI: Himedia, India) agar supplemented with 10% horse serum (Gibco-Invitrogen, USA) and 0.005 μg ml^{-1} amphotericin B (Sigma, USA) and then incubated under micro-aerobic conditions at 37°C for 72 hour. These indicator strains were obtained from the Department of Medical Science, Ministry of Public Health, Thailand. The clinical isolate of *H. pylori* (H40) was kindly provided by Dr. Thanittha Chutsuwan, Chulalongkorn University, Thailand. *Lactobacillus reuteri* ATCC 55730, a reuterin-producing strain used as a positive control, was cultured in MRS agar plates (Oxoid, Basingstoke, Hampshire, UK) at 37°C for 24-48 hour under anaerobic conditions.

Detection of reuterin production in Lactobacillus isolates using the spot overlay technique

Reuterin-producing strains of *Lactobacillus* were detected using a modification of the method described by Rosander et al[18]. Two hundred *Lactobacillus* isolates were tested for reuterin production. *L. reuteri* ATCC 55730, a reuterin-producing strain was used as a positive control with *Escherichia coli* ATCC 25922 and MRS used as negative controls. Each strain of *Lactobacillus* was spotted onto the surface of BHI agar supplemented with 20 mM glucose. *Lactobacillus* spots were allowed to develop under anaerobic conditions at 37°C for 24 hour. Soft agar (0.9% agar, 2% glycerol) was overlaid over the surface of each *Lactobacillus* plate and then incubated anaerobically at 37°C for 3 hour. The plates were then flooded with 2, 4-dinitro-phenylhydrazine. After 5 minutes, the 2, 4-dinitrophenylhydrazine was discarded in a waste container and KOH solution was added to the plate. The plate was then observed for a reddish brown color to develop, indicating reuterin production. KOH solution was then discarded as a hazardous chemical waste. These experiments were performed three times.

Preparation of water-glycerol supernatants and colorimetric assay of reuterin production

Overnight cultures of *Lactobacillus* were collected by centrifugation at 4,000x g for 10 minute at 4°C. Then, the cells were washed twice with sodium phosphate buffer, centrifuged again, and re-suspended in glycerol solution, followed by incubation under anaerobic conditions at 37°C for 3 hour. The cells were pelleted and supernatant containing reuterin was collected. The supernatant was passed through a syringe filter and stored at 4°C. Reuterin production was confirmed using an adapted method from Circle et al[19]. This assay is based on the colorimetric

determination of the acrolein formed from 3-HPA (reuterin) produced by glycerol dehydratase. For reuterin detection, a 20 µl sample of each supernatant was mixed with 15 µl of 10 mM L-tryptophan solution (Sigma, USA) and 60 µl of concentrated HCl. The mixtures were incubated for 20 minute at 37°C and the mixtures were closely observed for yellow color indicating the presence of reuterin.

Co-incubation of reuterin-producing strains with Escherichia coli ATCC 25922

Following Kinova Sepova's method[20], isolated reuterin-producing strains were co-incubated with *Escherichia coli* ATCC 25922. Briefly, overnight cultures of *Lactobacillus* and *E. coli* were pelleted and washed with potassium phosphate buffer twice and adjusted to 10^8 cells ml^{-1}. Equal portions were re-suspended in glycerol solution, followed by incubation under anaerobic conditions at 37°C for 3 hour. The cells were pelleted and supernatant collected and passed through a syringe filter.

Antimicrobial activities of reuterin against bacterial pathogens

Agar well diffusion assay[21] was used to determine the antimicrobial activity of reuterin in supernatants of water-glycerol solution against various pathogenic microorganisms: approximately $1x10^8$ cells ml^{-1} overnight cultures of 7 pathogens with the exception of *H. pylori* at $1x10^9$ cells ml^{-1} Each mixture was swabbed onto a plate. Circular wells of 4 mm diameter were made in the agar using a sterile cork borer and filled with reuterin in supernatants of water-glycerol solution. A solution of water-glycerol without reuterin was used as negative control. Supernatants of a reuterin-producing strain was used as a positive control. The plates were incubated for 24 hour or 72 hour at appropriate conditions for pathogens described above. The inhibition zone, a clear zone around the well showing no growth of pathogens, was measured and recorded in mm. at the center of the well. Three independent experiments, each done in duplicate, were performed; all data were expressed as mean $\pm$ SD.

Acid and bile tolerance test

Selected probiotic *Lactobacillus* was grown in MRS broth for 24 hour at 37°C anaerobically and diluted to an initial bacterial concentration of 10^8 cells ml^{-1}. The candidate strain was added to 10 ml of MRS broth (pH 6.5) or broth adjusted to pH 2, 3, 4, and 1%, 2%, 3%, and 4% bovine bile (Sigma, USA), respectively, and was incubated at 37°C for 3 hour under anaerobic conditions. Each solution was sampled and serial 10-fold dilutions in PBS (pH 7.2) of each sample were prepared (10^{-1}-10^{-5}). Viable bacterial counts were determined by spreading 100 µl of each serial dilution onto MRS agar plates and incubated anaerobically for 24-48 hour at 37°C. Viable bacteria were displayed as cell numbers of the log 10 of colonies grown on MRS agar compared to the initial bacterial concentration. All experiments were done in duplicate.

Identification of selected reuterin-producing isolates

For identification of *Lactobacillus* strain on the species level, parts of the 16S ribosomal DNA were amplified using polymerase chain reaction (PCR) and sequencing of PCR products were used. The template DNA for sequencing of the 16S ribosomal gene was forward primers 20F 5′AGTTTGATCCTGGCTC 3′ and reverse primers 1500R 5′ AAGGAGGTGATCCAGCC 3′. Briefly, a colony of each *Lactobacillus* overnight culture was re-suspened in 30 µl of milliQ water. Bacteria were lysed by sonication and DNA was purified. PCR was used to amplify the desired gene and the PCR products were purified using Geneaid Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Bade City, Taiwan). Sequence analysis of PCR products was performed by Macrogen in Seoul, Korea. The nucleotide sequences were processed using BioEdit and compared with sequences deposited in the Ribosomal Database Project (RDP-II; http://rdp.cme.msu.edu). The identity of the strain was determined based on the highest scores. The closest relatives based on 16S ribosomal gene sequences were evaluated, and a similarity of $\geq$98% to 16S ribosomal gene sequences of type strains was used as the criterion for identification.

Results

Selection of lactobacilli

Two hundred strains of lactobacilli were isolated from infant feces and presumptively screened using Gram staining and catalase testing. All isolates were Gram-positive and catalase-negative. Microscopic examination of isolates revealed variations including long and slender rods to short rods and coccobacilli. Bacterial colonies were generally small to medium (1-2 mm) with white circular, convex, and smooth morphologies.

Production of reuterin

Using the spot overlay method, the strain MSMC64-1 was identified as a reuterin producer. When

compared to *L. reuteri* ATCC 55730, MSMC64-1 also displayed reddish brown coloration around its spot. The colorimetric assay confirmed the presence of reuterin.

Antimicrobial activity of reuterin

The antimicrobial activity of strain MSMC64-1 supernatants in water-glycerol solution against pathogenic microorganisms is shown in Fig. 1. The results indicated that supernatants containing reuterin from strain MSMC64-1 showed inhibitory activities against all target pathogens. There were very strong inhibitory activities with a clear zone of 31 mm and 24 mm against *S. typhi* DMST 5784 and methicillin resistant *S. aureus* (MRSA) DMST 20651, respectively. There was strong inhibitory activity against methicillin resistant *S. aureus* (MRSA) DMST 20654, *V. parahaemolyticus* DMST 5665 and *S. dysenteriae* DMST 15111 with a clear zone of 21 mm, 19 mm, and 17 mm, respectively. Moderate to weak inhibitory activities against *V. cholera* DMST 2873 and *H. pylori* (H40) with a clear zone of 9 mm (Fig. 1) were observed. The water-glycerol solution (negative control) showed no inhibitory effect on any of the pathogenic strains tested.

Co-incubation of Lactobacillus and Escherichia coli

After prolonged (>six passages) monoculture, *Lactobacillus* isolates and the positive control *L. reuteri* ATCC 55730 showed markedly reduced reuterin production and lack of antimicrobial activity in repeat assays. Co-incubation with *Escherichia coli* prompted reuterin production and subsequent passages of pure *Lactobacillus* culture supernatants showed reproducible antimicrobial activity.

Acid and bile tolerance

The result of acid tolerance testing (survival at various pH values) showed that the viability of MSMC64-1 changed after incubation for 3 h at pH 2, 3, and 4, respectively. MSMC64-1 isolate could survive after incubation for 3 h at various pH values and bile conditions. The viable counts at pH 2, 3, and 4 decreased by about 4, 1, and 1 log values, respectively (Fig. 2). In the bile tolerance test, the viability of the tested isolate changed after incubation for 3 hour at 1-4% bile. Viable counts at 1%, 2%, and 3% bile, increased by about 1 log values, but in 4% bile, bacterial counts showed no log difference (Fig. 3). All values were compared with MRS bacteria media control. In particular, strain MSMC64-1 tolerated acid and bile

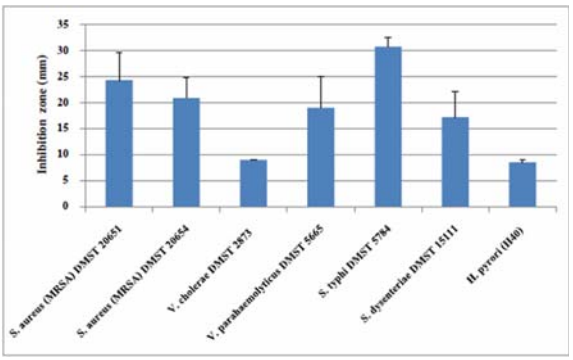

Fig. 1 Antagonistic activities of MSMC64-1 strain against selected bacterial pathogens.

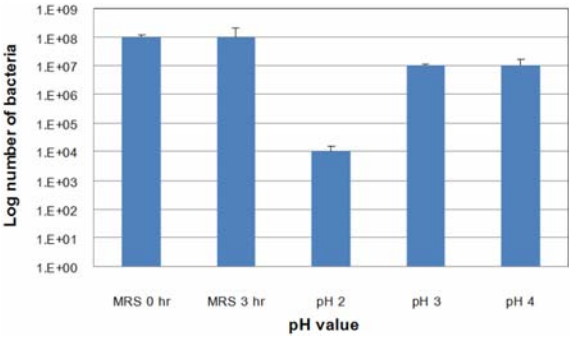

Fig. 2 Survival of MSMC64-1 strain after 3 hour of incubation with MRS broth at pH 2.0, 3.0, and 4.0.

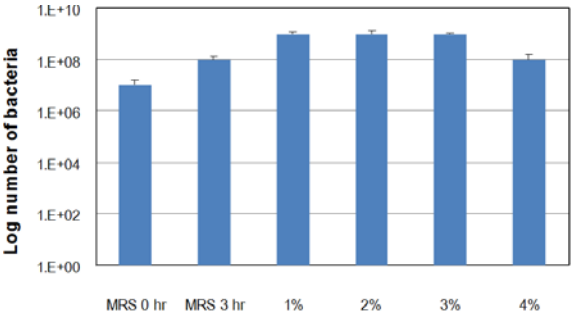

Fig. 3 Survival of MSMC64-1 strain after 3 hour of incubation with MRS broth supplemented with 1%, 2%, 3%, and 4% bile.

conditions.

Identification of Lactobacillus strain

PCR amplification of purified bacterial DNA from the strain of interest resulted in products approximately 1,500 bp in size visualized using gel electrophoresis. The products were subsequently purified and 16s ribosomal DNA sequencing of isolate MSMC64-1 was done. Comparison of the 16s ribosomal

DNA sequence with the Ribosomal Database Project revealed 98% sequence homology with *Lactobacillus reuteri*.

Discussion

Probiotic lactobacilli are commonly known to have health promoting attributes, including antimicrobial actions that can inhibit intestinal and food-borne pathogens[4,5,22]. In the present study, 200 isolates of lactobacilli were isolated from infant feces, of which 1 strain produced reuterin, a broad spectrum antimicrobial compound produced during anaerobic fermentation of glycerol by the action of glycerol[9,13,23]. This was detectable by both spot overlay and colorimetric methods, demonstrating the capability of *L. reuteri* MSMC64-1 to retain and accumulate reuterin and was selected for further study.

These findings are consistent with other studies that have demonstrated strain-specific reuterin production and accumulation against a broad-spectrum of Gram-positive, Gram-negative bacteria, yeast, fungi, protozoa and viruses[12,13,23,24]. Some strains of *L. reuteri* did not produce reuterin as avidly[24] while one study of *L. reuteri* BPL-36 reported activity against *Pseudomonas aeruginosa*, *E. coli* O157: H7, and *Listeria monocytogenes*[15].

A number of factors appear to influence the production of reuterin: a study found co-incubation of *L. reuteri* ATCC 55730 and *E. coli* induced and increased reuterin production[20]. Without competing micro-organisms, no evolutionary beneficial reason exists for *L. reuteri* to produce reuterin. Instead, metabolic processes may be saved for tasks that are more important. This could explain why both *L. reuteri* MSMC64-1 and *L. reuteri* 55730 showed decreased reuterin production in monoculture. Chung et al[23] reported that reuterin production by *L. reuteri* significantly increased in the presence of *E. coli*.

Reuterin can also be produced by *Lactobacillus* species such as *L. brevis*, *L. collinoides*[25] and *L. coryniformis*[12]. Among these species, certain strains of *L. reuteri* is reported to produce relatively high amounts of reuterin[10,26]. As a major *Lactobacillus* species found in many animal hosts[27], it was not surprising, therefore, that molecular analysis of our strain was consistent with *L. reuteri*.

One important characteristic of probiotic bacteria is the ability to survive under the effects of acid and bile in the host gastrointestinal tracts and exert desired effects at the desired location. *L. reuteri* MSMC64-1 showed the ability to survive under acid and bile conditions. Different *L. reuteri* strains appears to have adapted to different animal hosts; therefore, *Lactobacillus* strains isolated from humans may be more easily adapted for use as human probiotics than isolates from other sources[28].

Conclusion

L. reuteri MSMC64-1 was assayed for reuterin production and antagonistic activities against enteric and other pathogens. The data showed that reuterin produced by *L. reuteri* MSMC64-1 is effective against most tested pathogenic microorganisms including *S. typhi* DMST 5784, methicillin-resisitant *S. aureus* DMST 20651, MRSA DMST 20654, *V. parahaemolyticus* DMST 5665, *S. dysenteriae* DMST 15111, *V. cholerae* DMST 2873 and *H. pylori* (H40).

Furthermore, *L. reuteri* MSMC64-1 showed the ability to survive under acid and bile conditions which are the important characteristics for survival in the gastrointestinal tract. With further characterization and in vivo testing, this *Lactobacillus* candidate strain may possibly be used as a human-derived, Thai probiotic strain with potential clinical and therapeutic relevance.

What is already known on this topic ?

Reuterin is a previously characterized antimicrobial substance produced by strains of *Lactobacillus*.

What this study adds ?

Our isolated strain, *L. reuteri* MSMC64-1, showed the ability to produce reuterin and tolerate acid and bile conditions, which are important characteristics of probiotics for survival in the gastrointestinal tract.

Acknowledgement

This work was funded by a research grant from the Faculty of Medicine and HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University. The authors thank the Department of Microbiology, Faculty of Medicine, Srinakharinwirot University for supporting equipment and facilities for our research.

Potential conflicts of interest

None.

References

1. WHO/FAO. Probiotics in food: Health and nutritional properties and guidelines for evaluation.

FAO Food and Nutrition paper 85. Rome: WHO/FAO; 2006.
2. Kailasapathy K, Chin J. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol 2000; 78: 80-8.
3. Johnson CL, Versalovic J. The human microbiome and its potential importance to pediatrics. Pediatrics 2012; 129: 950-60.
4. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. Cochrane Database Syst Rev 2010; (11): CD003048.
5. Rohde CL, Bartolini V, Jones N. The use of probiotics in the prevention and treatment of antibiotic-associated diarrhea with special interest in *Clostridium difficile*-associated diarrhea. Nutr Clin Pract 2009; 24: 33-40.
6. Bermudez-Brito M, Plaza-Diaz J, Munoz-Quezada S, Gomez-Llorente C, Gil A. Probiotic mechanisms of action. Ann Nutr Metab 2012; 61: 160-74.
7. Gibson MK, Pesesky MW, Dantas G. The yin and yang of bacterial resilience in the human gut microbiota. J Mol Biol 2014; 426: 3866-76.
8. Talarico TL, Dobrogosz WJ. Chemical characterization of an antimicrobial substance produced by *Lactobacillus reuteri*. Antimicrob Agents Chemother 1989; 33: 674-9.
9. Talarico TL, Casas IA, Chung TC, Dobrogosz WJ. Production and isolation of reuterin, a growth inhibitor produced by *Lactobacillus reuteri*. Antimicrob Agents Chemother 1988; 32: 1854-8.
10. Vollenweider S, Grassi G, Konig I, Puhan Z. Purification and structural characterization of 3-hydroxypropionaldehyde and its derivatives. J Agric Food Chem 2003; 51: 3287-93.
11. Wise C, Novitsky L, Tsopmo A, Avis TJ. Production and antimicrobial activity of 3-hydroxypropionaldehyde from *Bacillus subtilis* strain CU12. J Chem Ecol 2012; 38: 1521-7.
12. Magnusson J, Strom K, Roos S, Sjogren J, Schnurer J. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. FEMS Microbiol Lett 2003; 219: 129-35.
13. Cleusix V, Lacroix C, Vollenweider S, Duboux M, Le Blay G. Inhibitory activity spectrum of reuterin produced by *Lactobacillus reuteri* against intestinal bacteria. BMC Microbiol 2007; 7: 101.
14. Bian L, Molan AL, Maddox I, Shu Q. Antimicrobial activity of *Lactobacillus reuteri* DPC16 supernatants against selected food borne pathogens. World J Microbiol Biotechnol 2011; 27: 991-8.
15. Mishra SK, Malik RK, Manju G, Gaare M, Pandey N, Singroha G, et al. Characterization of a Reuterin-Producing *Lactobacillus reuteri* BPL-36 Strain Isolated from Human Infant Fecal Sample. Probiotics Antimicrob Proteins 2012; 4: 154-61.
16. Vollenweider S, Evers S, Zurbriggen K, Lacroix C. Unraveling the hydroxypropionaldehyde (HPA) system: an active antimicrobial agent against human pathogens. J Agric Food Chem 2010; 58: 10315-22.
17. Schaefer L, Auchtung TA, Hermans KE, Whitehead D, Borhan B, Britton RA. The antimicrobial compound reuterin (3-hydroxypropionaldehyde) induces oxidative stress via interaction with thiol groups. Microbiology 2010; 156: 1589-99.
18. Rosander A, Connolly E, Roos S. Removal of antibiotic resistance gene-carrying plasmids from *Lactobacillus reuteri* ATCC 55730 and characterization of the resulting daughter strain, *L. reuteri* DSM 17938. Appl Environ Microbiol 2008; 74: 6032-40.
19. Circle SJ, Stone L, Boruff CS. Acrolein determination by means of tryptophane. A colorimetric micromethod. Ind Eng Chem Anal Ed 1945; 17: 259-62.
20. Kinova Sepova H, Bilkova A. Isolation and identification of new lactobacilli from goatling stomach and investigation of reuterin production in *Lactobacillus reuteri* strains. Folia Microbiol (Praha) 2013; 58: 33-8.
21. Chung KT, Thomasson WR, Wu-Yuan CD. Growth inhibition of selected food-borne bacteria, particularly *Listeria monocytogenes*, by plant extracts. J Appl Bacteriol 1990; 69: 498-503.
22. Hickson M. Probiotics in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* infection. Therap Adv Gastroenterol 2011; 4: 185-97.
23. Chung TC, Axelsson L, Lindgren SE, Dobrogosz WJ. In vitro studies on reuterin synthesis by *Lactobacillus reuteri*. Microb Ecol Health Dis 1989; 2: 137-44.
24. Spinler JK, Taweechotipatr M, Rognerud CL, Ou CN, Tumwasorn S, Versalovic J. Human-derived probiotic *Lactobacillus reuteri* demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. Anaerobe 2008; 14: 166-171.
25. Claisse O, Lonvaud-Funel A. Assimilation of glycerol by a strain of *Lactobacillus collinoides* isolated from cider. Food Microbiol 2000; 17: 513-9.
26. Kang MS, Oh JS, Lee HC, Lim HS, Lee SW, Yang

KH, et al. Inhibitory effect of *Lactobacillus reuteri* on periodontopathic and cariogenic bacteria. J Microbiol 2011; 49: 193-9.
27. Casas IA, Dobrogosz W. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. Microb Ecol Health Dis 2000; 12: 247-85.
28. Spinler JK, SontakkeA, Hollister EB, Venable SF, Oh PL, Balderas MA, et al. From prediction to function using evolutionary genomics: human-specific ecotypes of *Lactobacillus reuteri* have diverse probiotic functions. Genome Biol Evol 2014; 6: 1772-89.

คุณสมบัติในการยับยั้งจุลินทรีย์ของเชื้อแลคโตบาซิลลัสที่มีศักยภาพเป็นโพรไบโอติกที่แยกได้จากอุจจาระของเด็กทารกไทย

จันทนา ฉิมแจ่ง, ตะลันต์ เทพอารีย์, บุญรัตน์ ลัดดา, สมบูรณ์ ธนาสุภวัฒน์, เบญจมาส ถนอมทรัพย์ วงศ์สัตยานนท์, มาลัย ทวีโชติภัทร์*

ภูมิหลัง: *โพรไบโอติกเป็นแนวทางการรักษาที่ได้รับความนิยมมากยิ่งขึ้น โดยเฉพาะการติดเชื้อในระบบทางเดินอาหารและโรคอุจจาระร่วงที่เกี่ยวข้องกับการใช้ยาปฏิชีวนะ แบคทีเรียซึ่งเป็นโพรไบโอติกหลายสายพันธุ์ถูกจัดให้อยู่ในสกุล Lactobacillus และสามารถพบได้ในระบบทางเดินอาหารของทารก*

วัตถุประสงค์: *การวิจัยนี้เพื่อตรวจสอบฤทธิ์การยับยั้งจุลินทรีย์ของเชื้อแลคโตบาซิลลัสจำนวน 200 ไอโซเลต ซึ่งแยกได้จากอุจจาระของเด็กทารกที่มีสุขภาพดี*

วัสดุและวิธีการ: *ตรวจหาการสร้างสารรอยเทอรินโดยวิธี spot overlay technique และ colorimetric assay ทดสอบหาฤทธิ์ยับยั้งจุลินทรีย์โดยวิธี agar well diffusion*

ผลการศึกษา: *พบว่าเชื้อแลคโตบาซิลลัสสายพันธุ์ MSMC64-1 สามารถสร้างรอยเทอรินและมีฤทธิ์ในการยับยั้งจุลินทรีย์ทดสอบทั้ง 7 ชนิด คือ Salmonella typhi DMST 5784 และ methicillin resistant Staphylococcus aureus (MRSA) DMST 20651 ได้ฤทธิ์แรงมาก รองลงมาคือ Staphylococcus aureus (MRSA) DMST 20654, Vibrio parahaemolyticus DMST 5665 และ Shigella dysenteriae DMST 15111 และมีฤทธิ์ในการยับยั้ง Vibrio cholerae DMST 2873 และ Helicobacter pylori (H40) ได้ในระดับน้อยถึงปานกลาง เชื้อแลคโตบาซิลลัสสายพันธุ์ MSMC64-1 ยังมีความสามารถทนต่อสภาวะกรดที่ระดับ pH2, 3, 4 และทนต่อเกลือน้ำดีที่ระดับความเข้มข้น 1%, 2%, 3% และ 4% เมื่อจำแนกชนิดของเชื้อโดยใช้เทคนิค 16S rDNA พบว่าจัดอยู่ใน Lactobacillus reuteri โดยมีความเหมือนของลำดับนิวคลิโอไทด์ที่ 98%*

สรุป: *เชื้อแลคโตบาซิลลัสที่แยกได้มีศักยภาพในการนำมาใช้เป็นโพรไบโอติกเพื่อป้องกันและรักษาโรคติดเชื้อในระบบทางเดินอาหาร*