World Journal of Advanced Research and Reviews, 2020, 05(03), 149–162

World Journal of Advanced Research and Reviews

e-ISSN: 2581-9615, Cross Ref DOI: 10.30574/wjarr

Journal homepage: https://www.wjarr.com

(RESEARCH ARTICLE)

Probiotic property and biological activities of *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 isolated from Thai population

Klungsupya Prapaipat [1,*], Saiki Papawee [2], Wannissorn Bhusita [1], Ruengsomwong Supatjaree [1], Muangman Thanchanok [1], Bamrungchue Nantana [1] and Taengphan Weerasak [1]

[1] *Research and Development Group for Bio-Industries, Thailand Institute of Scientific and Technological Research (TISTR), Techno Polis, Khlong Luang, Pathum Thani 12120, Thailand.*
[2] *Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8566, Japan.*

Publication history: Received on 20 March 2020; revised on 26 March 2020; accepted on 28 March 2020

Article DOI: https://doi.org/10.30574/wjarr.2020.5.3.0072

Abstract

Bifidobacterium animalis subsp. *lactis* TISTR 2591(*B. L* TISTR) isolated from Thai newborn babies and identified regarding 16S rDNA analysis. Its probiotic property was assessed on hemolytic activity, antibiotic susceptibility, gastric acid and bile resistance capacity, antimicrobial activity and adhesion ability. The biological properties of *B. L* TISTR were evaluated on blood metabolic parameters (cholesterol, glucose and insulin levels) and metabolic genes expression. The obtained results indicated that *B. L* TIST*R* has no hemolytic activity. Its adhesion ability to intestinal epithelium of Caco-2 and HT-29 cell lines is greater than that of the two commercial strains (*B. lactis* and *L. Reuteri*). No antibiotic resistance property of *B. L* TISTR was found on eight antibiotics tested. Moreover, it possesses antimicrobial activity against twelve pathogenic bacteria covering seven intestinal pathogens, two oral pathogens and three skin pathogens. It was found that *B. L* TISTR could induce insulin sensitivity in adipose tissue that was related with reducing glucose. Also, it was shown that cholesterol level in plasma *B. L* TISTR fed mice decreased significantly. Expression of three metabolic genes including *Srebp-1c*, *Glut4* and *Ucp1* was found. These three genes are involved in gluconeogenic control, insulin sensitivity and cholesterol and fatty acid metabolism, respectively. We examined *Il10* and *Nos*2 expression in Peyer's patches from intestinal and found an induction of *Il10* expression. We also found that *Nos2* expression decreased in *B. L* TISTR fed mice. These observations suggest that B. L TISTR may improve immune system and reduce inflammation activity which in turn providing prevention of intestinal inflammation.

Keywords: *Bifidobacterium animalis* subsp. *lactis* TISTR 2591; Probiotic; Glucose; Insulin; Cholesterol.

1. Introduction

According to a report by Grand View Research, the global market for functional foods in the coming years is predicted to grow rapidly and may reach USD 275.77 billion by 2025 [Grand View Research. https://www.prnewswire.com/news-releases/the-global-functional-foods-market-size-is-projected-to-reach-usd-275-77-billion-by-2025--300841184.html. Last accessed on 19/03/2020]. The fastest rate of growth is expected to be in the United States, although Japan currently accounts for about one-half of this market. Probiotic products represent a strong growth area within the functional foods group and intense research efforts are under way to develop dairy products into which probiotic organisms such as Lactobacillus and Bifidobacterium species are incorporated. According to the Food and Agriculture Organization of the United Nations World Health Organization (FAO/WHO) [1], probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host. In relation to food, Lilly and Stillwell [2] stated that the definition of probiotic could be adjusted by emphasizing that the beneficial effect was exerted by the microorganisms when consumed in adequate amounts as part of food. It is well accepted that probiotics are gaining in popularity because of their health benefits. Many research studies point to the

* Corresponding author: Klungsupya Prapaipat

benefits of probiotic use which include digestive health by Elizabeth and Susan [3], immune health by Yan and Polk [4], women's health by Reid and and De Alberti *et al*., [5, 6], inflammatory disorders and anticancer effects by Kumar *et al*., [7]. However, other study by Figueroa-González *et al*., [8] indicated a wide variety of other emerging health benefits continue to be uncovered. It is important to note that health benefits provided by probiotics are strain specific and not species- or genus-specific. Therefore, no single probiotic strain can provide all proposed benefits, not even strains of the same species and not all strains of the same species will be effective against defined healthy conditions.

Holzapfel *et al*., [9] reported that most of probiotic microorganisms belong to the genera Lactobacillus and Bifidobacterium. In 2008, Mercenier *et al*., [10] demonstrated a difference of Bifidobacterium from Latobacillus due to its unique metabolic pathway called bifid shunt. Findings of Senok *et al*., [11], de Vrese and Schrezenmeir [12] and Kaur *et al*., [13] indicated that among many Bifidobacterium strains, the most commonly used as probiotics are *B. longum*, *B. bifidum*, *B. infantis*, *B. lactis*, *B. breve*, *B, adolescentis*, *B. lactis* and *B. animalis*. Proven effects of Bifidobacteria on human health were reported. In 1999, Gomes and Malcata [14] reported that *B. breve* could activate the humoral immune system by augmenting anti-rotavirus IgA production or anti-influenza virus, adhere to human intestinal epithelial cells and inhibit enteropathogen-cell interactions. Effectiveness of *B bifidum* in mediating the clinical course of murine rotavirus diarrhea was initiated in BALB/c lactating mice and their litters by Duffy *et al*., [15, 16]. It demonstrated that treatment of *B. bifidum* significantly reduced murine rotavirus (MRV) diarrhea within 2 to 10 days after inoculation. They found that *B. bifidum* showed competitive exclusion activity against pathogenic or putrefactive bacteria such as *Escherichia coli* and *Candida* spp., was able to reduce the incidence of diarrhea by rotavirus. *B. animalis* normalizes the large intestines of most mammals, including humans. It reduced the risk of acute diarrhea in children and adults. The recent clinical study reported by Abou El-Soud *et al*., [17] confirmed the preventive activity against diarrhea. They concluded that probiotics in supplemented milk formula decreased significantly frequency, duration of diarrhea and hospital stay than usual treatment alone in children with acute diarrhea. Some Bifidobacteria are currently and widely used in commercial products such as dairy products and dietary supplements. Based on results of clinical studies, several companies have invented scientific-sounding names for the strains. For examples, *B. lactis* Bl-04 and B1-07 from DuPont's Danisco FloraFIT, *B. lactis* HN019 known as HOWARU Bifido from Fonterra licensed to DuPont, *Bifidobacterium animalis* DN-173 010 from Danone, France. *B. longum* BB536 from Snow Brand Milk Products, Japan and *B. animalis* subsp. *lactis* BB-12 from Chr. Hansen, USA. [18, 19].

In Thailand, the utilization of probiotics in consumer packaged goods has expanded in recent years from food (e.g. yogurt) and health care (e.g. dietary supplements) to beverages and personal care products. It appears that consumers have better understanding on various functional benefits of probiotics. Since 2012, the Ministry of Public Health announced the Food Notification (no.346) on Use of Probiotic Microorganisms in Foods. It is stated that the probiotic containing products shall be approved by Thai Food and Drug Administration (Thai FDA) before commercialization, only microorganisms specified in an Annex of this notification are allowed to be used as probiotics (23 probiotic are currently on the list) and viable probiotic microorganisms in such food must not less than 1,000,000 CFU/1 g food at the end of its shelf life. The Biodiversity Research Centre (BRC) of Thailand Institute of Scientific and Technological Research (TISTR) is responsible for culture collection of bacteria, yeasts, molds and microalgae which are beneficial to industrial and environmental usage. In the case of probiotic microorganisms, BRC has more than 500 probiotics isolated from various samples including local food, natural fauna and flora, specimens form Thai volunteers etc. Among these 500 probiotic strains, *B. animalis* subsp. *lactis* TISTR 2591 was selected and investigated on its probiotic property as well as biological activities to determine its health benefit effects. The data obtained from this study would be the first scientific information to support and promote utilisation of *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 as the novel potential probiotic for industrially used in food and functional food products.

2. Material and methods

2.1. Bacterial strain and culture

B. animalis subsp. *lactis* TISTR 2591 (*B. L* TISTR) was previously isolated from Thai newborn baby under the project approved by the human research ethic committee of Thammasart University, Faculty of Medicine, Thammasart University, Bangkok, Thailand with the approval No. MTU-EC-OB-3-039/59. Analysis of 16S rDNA using 27F primer (5´ GAGTTTGATCATGGCTCAG3´) and 1492R primer (5´CGGTTACCTTGTTACGACTT3´) revealed that this bacterium was *B. animalis* subsp. *lactis* by 99% homology (http://www.ncbi.nlm.nih.gov/). The *B. L* TISTR was grown anaerobically in Man Rogosa Sharpe (MRS) broth (Merck, Germany) containing 0.05% L-Cysteine HCl (Merck KGaA, Germany). The culture was stored in 40% glycerol and kept at - 80 ℃.

2.2. Hemolytic activity

B. L TISTR was streaked on 5% sheep blood agar (SBA) and incubated anaerobically at 37 ℃ for 24 hours. After incubation, hemolytic zone around the colonies were observed.

2.3. Antibiotic susceptibility test

Minimal inhibitory concentration (MIC) values of eight antibiotic types was determined using commercial MIC Test strips (Liofilchem srl, Italy) including ampicillin, chloramphenicol, tetracycline, erythromycin, clindamycin, vancomycin, streptomycin, and gentamycin, respectively. The bacterium was grown anaerobically in MRS broth containing 0.05% L-Cysteine HCl for 24 hours. To prepare the inoculum, the turbidity of the overnight culture was adjusted with normal saline to match that of McFarland standard No. 0.5. The inoculum was applied on Iso-Sensitest agar (Oxoid, UK) by cotton swab. Then each MIC strip was laid over the inoculated agar. The MIC values were read after 24 hours anaerobic incubation at 37 ℃. Based on EFSA 2012 guideline, the MIC vales was interpreted and reported as S (sensitive) or R (resistance).

2.4. Determination of survival in simulated gastric juice and simulated intestinal juice

Tolerance to simulated gastric juice and simulated intestinal juice was performed in accordance with the method by Charteris *et al*., [20] with slight modification. Cell pellets of *B. L* TISTR were prepared by centrifuging 1 ml of the 24 hours old culture grown anaerobically in MRS broth containing 0.05% L-Cysteine HCl at 7,000 rpm for 5 minutes. The pellets were collected and washed twice with phosphate buffered saline (PBS). For survival assay, the cell pellets were suspended in 1 ml of simulated intestinal juice (0.85% NaCl and 0.3% pepsin) pH 2 and incubated anaerobically at 37 ℃. The concentration of bacterial cells was counted at 0 and 90 minutes, respectively. Percentage of cell survival was calculated as following

$$\% \text{ survival} = \frac{B \times 100}{A}$$

When A = average amount of bacteria counted at 0 minute

B = average amount of bacteria counted at 90 minutes

The experiment was repeated with simulated intestinal juice (0.85% NaCl, 0.3% bile salt and 0.1 % pancreatin) pH 8.

2.5. Antimicrobial activity assay

Cell free supernatant was prepared by growing *B. L* TISTR anaerobically in MRS broth containing 0.05% L-Cysteine HCl at 37 ℃ for 48 hours. The supernatant was collected by centrifugation at 7,000 rpm for 10 minutes and then was filtered sterilized through 0.22 μm millipore membranes (Sartorius, UK). Pathogenic bacteria used in this study together with their growth media and conditions were shown in Table 1.

Assay of the antimicrobial activity was carried out using agar well diffusion method described by Balouiri *et al*., [21]. The inoculum of each pathogenic bacterium was prepared by growing the pathogen on the suitable medium (Table 1). After incubation, a few single colonies were suspended in normal saline solution followed by adjusting the turbidity to match that of McFarland standard No. 0.5. The test agar for each pathogenic bacterium was prepared by applying the inoculum over the surface of medium. Then, a hole of 6 mm were made at the center of test agar plate. Seventy microliters of cell free supernatant were loaded into each well. After appropriate incubation, sizes of clear zones observed around the wells were recorded and reported in mm.

Assay of the antimicrobial activity was carried out using agar well diffusion method described by Balouiri *et a*l., [21]. The inoculum of each pathogenic bacterium was prepared by growing the pathogen on the suitable medium (Table 1). After incubation, a few single colonies were suspended in normal saline solution followed by adjusting the turbidity to match that of McFarland standard No. 0.5. The test agar for each pathogenic bacterium was prepared by applying the inoculum over the surface of medium. Then, a hole of 6 mm were made at the center of test agar plate. Seventy microliters of cell free supernatant were loaded into each well. After appropriate incubation, sizes of clear zones observed around the wells were recorded and reported in mm.

Table 1 Pathogenic bacteria used, growth media and growth conditions.

Bacterial strains	Medium used	Incubation conditions
Escherichai coli ATCC 8379	MHA	37 ℃; 24 hours
Salmonella typhimurium ATCC 11331	MHA	37 ℃; 24 hours
Salmonella enteritidis DMST 15676	MHA	37 ℃; 24 hours
Listeria monocytogenes DMST 13802	MHA	37 ℃; 24 hours
Staphylococcus aureus ATCC 6538	MHA	37 ℃; 24 hours
Bacillus cereus ATCC 11778	MHA	37 ℃; 24 hours
Helicobacter pylori PT4 (clinical isolate)	7% SBA	37 ℃; 24 hours, microaerophillic
Clostridium perfringens DMST 17112	5% SBA	37 ℃; 24 hours, anaerobic
Streptococcus mutans ATCC 25175	5% SBA	37 ℃; 24 hours, anaerobic
Streptococcu sobrinus ATCC 27351	5% SBA	37 ℃; 24 hours, anaerobic
Propionibacterium acnes DMST 14961	5% SBA	37 ℃; 24 - 48 hours, anaerobic
Staphylococcus epidermidis TISTR518	MHA	37 ℃; 24 hours

Note: TISTR = TISTR culture collection Thailand: ATCC= American Type Culture Collection, USA: DMST = National Institute of Health of Thailand, Department of Medical Science, Thailand: Clinical isolate kindly provided by Department of Surgery, Faculty of Medicine, Thammasart University, Thailand. MHA = Mueller Hinton Agar

2.6. Determination of adhesion ability

In vitro adhesion ability of *B. L* TISTR was carried out with 2 types of cell line, Caco-2 ATCC® HTB37™ and HT-29 ATCC® HTB38™. The Caco-2 cell line was grown in Dulbecco's modified Eagle's minimal essential medium (DMEM)-High glucose w/L-Glutamine w/Sodium pyruvate (Biowest, USA), while HT-29 cell line was grown using Roswell Park Memorial Institute (RPMI 1640 w/L-Glutamine w/25mM Hepes (Biowest, USA). Both media were supplemented with 10% heated inactivated fetal bovine serum (Gibco, USA) and 1% antibiotic - antimycotic solution (Gibco, USA) in 24-well culture plate and incubated at 37 ℃ under 5% CO_2 for 21 days. The media were change every 3 - 4 days. To prepare the bacterial pellets, 24 hours anaerobically grown of *B. L* TISTR in MRS broth containing 0.05% L-Cysteine HCl was centrifuged at 7000 rpm for 10 minutes. The cell pellets were collected and washed 3 times with normal saline solution (NSS) before being resuspended with DMEM or RPMI 1640 without antibiotics to the approximate concentration of 10^8 cfu/ml. The concentration of bacterial cells was confirmed by viable plate count. The bacterial cell suspension was kept at 4 ℃ and used within 1 hour. Prior to the assay, the DMEM medium was gently removed from each well. The confluent monolayer was washed twice with Dulbecco PBS. Then, 2 ml of DMEM without antibiotics were added into each well. Incubation was done at 37 ℃ under 5 % CO_2 for 1 hour. At the end of incubation time, the DMEM were removed and 1 ml of the bacterial cell suspension was added into each well. Following incubation under the same conditions for 1 hour, the bacterial cells were removed and each well was washed 3 times with Dulbecco PBS. The amount of the attached bacteria was determined by cell viable count and used for calculating % adhesion compared to the inoculum cell concentration.

2.7. Animal and study design

Twelve male C57BL/6 mice (Japan SLC, Inc., Shizuoka, Japan) of 12-week old were employed. They were divided into the control and treatment groups and respectively fed with an AIN93M formula diet (Oriental Yeast Co. Ltd., Tokyo, Japan) and the diet mixed with 0.5% (w/w) freeze dried *B. L* TISTR for seven weeks. Mice were sacrificed after 16 hours fasting. Blood and tissues were collected. Tissues were rapidly frozen in liquid nitrogen. The animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals at the National Institute of AIST, and the Animal Care and Use Committee at AIST approved the study protocol (Approval no. 2016 - 054F).

2.7.1. Determination of blood metabolic parameters

Mouse blood was collected in EDTA-2Na coated tubes (Terumo Corporation, Tokyo, Japan) was immediately separated by centrifugation at 3000 rpm for 10 minutes at 4 ℃. Platelet-poor plasma was collected and stored at -20 ℃. Glucose

level in plasma was determined by LabAssay™ Glucose (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Cholesterol levels in plasma was measured using LabAssay™ Cholesterol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Insulin level in plasma was determined by Mouse Insulin ELISA Kit (Shibayagi Co., Ltd., Gunma, Japan).

2.7.2. Determination of metabolic genes expression by real-time quantitative PCR

White adipose tissue (WAT), brown adipose tissue (BAT), liver and Peyer's patches from intestine were collected and determined on genes expression. Total RNA was extracted from the cells using an RNAiso plus (total RNA extraction reagent). Single-stranded cDNA was generated using the PrimeScript™ RT Master Mix. Quantitative real-time PCR was carried out using a SYBR® Premix Ex Taq™ II (Takara Bio. Inc., Otsu, Japan) and a LightCycler™ (Roche Diagnostics, Mannheim, Germany). All primer sequences were purchased from Thermo Fisher Scientific Inc. (Massachusetts, USA). The primer sequences are listed in Table 2. The PCR conditions were 95 ℃ for 10s followed by 45 cycles of 95 °C for 5 seconds, 58 °C for 10 seconds and at 72 °C for 10 seconds. The amount of target mRNA was normalized relative to the internal standard Gapdh [22].

Table 2 Primer sequences for real time reverse transcription (RT)-PCR.

Target gene	Direction	Primer sequences (5′→ 3′)
Srebp-1c	Forward	ATCGGCGCGGAAGCTGTCGGGGTAGCGTC
	Reverse	ACTGTCTTGGTTGTTGATGAGCTGGAGCAT
Glut4	Forward	CTGTCGCTGGTTTCTCCAAC
	Reverse	CAGGAGGACGGCAAATAGAA
Ucp1	Forward	GGCAACAAGAGCTGACAGTAAAT
	Reverse	GGCCCTTGTAAACAACAAAATAC
Cyp7a1	Forward	AGCAACTAAACAACCTGCCAGTACTA
	Reverse	GTCCGGATATTCAAGGATGCA
Pparα	Forward	TGCAAACTTGGACTTGAACG
	Reverse	AGGAGGACAGCATCGTGAAG
G6pc	Forward	TGGTAGCCCTGTCTTTCTTTG
	Reverse	TTCCAGCATTCACACTTTCCT
Gck	Forward	GATCCGGGAAGAGAAGCAAG
	Reverse	GACAGGGATGAGGGACAGAG
Il10	Forward	GACAACATACTGCTAACCGACTCC
	Reverse	GCTCCTTGATTTCTGGGCCATG
Nos2	Forward	CATTCTACTACTACCAGATCGAGCC
	Reverse	CACACACTTTCACCAAGACTCTAAAT

2.7.3. Statistical Analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) with Dunnett's test for selected pairs. The statistical software "EZR" was used for statistical analysis [23].Values are indicated as means ± SE. The significant differences are shown as probability values.

3. Results

3.1. Morphological and cultural characteristics of the isolate

Though *B. animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR) was previously isolated from faeces (meconium) obtained from the 3 day-old infants and already identified using 16S rDNA analysis. This bacterium had not been studied for its probiotic activities before. In this study, the probiotic properties in term of safety and ability to survive in harsh

conditions according to WHO/FAO 2002 Guideline were carried out. As illustrated in Figure 1, the colony of *B. L* TISTR grown on MRS agar plate produced a small, white, circular, raised and shiny appearance with irregular edges. Gram staining illustrated Gram-positive with typical cell shape of Bifidobacterium.

Figure 1 Colony (left) and cells (right) of *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR).

3.2. Antibiotic susceptibility test

Apart from no hemolytic activity, *B. L* TISTR was susceptible to all eight antibiotics tested as shown in Table 3.

Table 3 Antibiotic susceptibility profile of *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR) compared to the commercial strains.

Antibiotic used	MIC values (μg/ml)		
	***B. animalis* subsp. *lactis* TISTR 2591 (*B. L TISTR*)**	***B. lactis*, commercial strain**	***L. reuteri*, commercial strain**
Ampicillin	0.023 (2)	0.094	6
Chloramphenicol	0.75 (4)	1	3
Vancomycin	0.38 (2)	0.75	>256
Tetracycline	1(8)	0.19	16
Erythromycin	0.023 (1)	0.094	0.38
Gentamicin	12 (64)	8	2
Streptomycin	12 (128)	2	32
Clindamycin	0.023 (1)	0.023	0.094

Note: The numbers in blanket = MIC cut off values according to EFSA, 2012.

3.3. Antimicrobial activity assay

Illustrated in Table 4, it could be seen that *B. L* TISTR possessed a wide broad spectrum of antibacterial activity. It exhibited inhibitory activity against all tested pathogenic bacteria including intestinal pathogens (*E. coli* ATCC 8379, *S. typhimurium* ATCC 11331, *L. monocytogenes* DMST 13802, *S. enteritidis* DMST 15676, *H. pyroli* PT4 clinical isolate, *Cl. perfringens* DMST17112 and *B. cereus* ATCC 117780), oral pathogens (*S. mutans* ATCC 25175 and *S. sobrinus* ATCC 27351) and skin pathogens (*S. epidermidis* TISTR 518 and *P. acnes* DMST 14961). *L. monocytogenes* DMST 13820 seemed to be the most susceptible while E. coli ATCC 8379 was the least susceptible to the cell free supernatant of *B. L* TISTR. It was found that *B. L* TISTR displayed greater antibacterial activity than the other two commercial probiotic strains of *B. lactis* and *L. reuteri*.

Table 4 Antimicrobial activity of cell free supernatant prepared from *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR)

Pathogenic bacteria	*Size of inhibition zone (mm)		
	B. L **TISTR**	***B. lactis*** **(commercial strain)**	***L. reuteri*** **(commercial strain)**
E. coli ATCC 8379	13	10	8
S. typhimurium ATCC 11331	19	9	19
L. monocytogenes DMST 13802	23	No clear zone	20
S. enteritidis DMST 15676	16	15	13
S. aureus ATCC 6538	20	No clear zone	18
S. epidermidis TISTR 518	17	8	15
P. acnes DMST 14961	12	No clear zone	No clear zone
S. mutans ATCC 25175	12	No clear zone	11
S. sobrinus ATCC 27351	14	No clear zone	No clear zone
H. pyroli PT4 (clinical isolate)	18	12	13
Cl. perfringens DMST 17112	20	10	10
B. cereus ATCC 11778	12	10	8

*Included diameter of well = 6 mm

3.4. Determination of survival in simulated gastric juice and simulated intestinal juice

It was shown that *B. L* TISTR could survive in simulated gastric juice pH 2 and in simulated intestinal juice containing 0.3% bile salt. After 90 minutes exposure to simulated gastric juice, 90.23% of *B. L* TISTR could survive under this test condition whereas simulated intestinal juice had no fatal effect on this bacterium (100% survival at 4 hours contact time).

3.5. Determination of adhesion ability

One important characteristic of probiotics is the ability in adhering to cells, especially epithelial cells along the intestinal intestine where probiotics live and have activities involved in health benefit of the host. In our study, *B. L* TISTR showed better adhesion ability to Caco-2 than to HT-29. Comparison between % adhesion of *B. L* TISTR and the other 2 commercial probiotic strains indicated that *B. L* TISTR may have better probiotic property than those commercial strains of *B. lactis* and *L. reuteri* (Table 5).

Table 5 Adhesion ability of *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR) to Caco-2 and HT-29 cell lines.

Test bacteria	% Mucosal adhesion	
	Caco-2	HT-29
B animalis subsp. *lactis* TISTR 2591 (*B. L* TISTR)	2.67	0.67
B. lactis commercial strain	0.35	0.29
L. reuteri commercial strain	0.75	0.89

3.6. Determination of Blood metabolic parameters

After free feeding of AIN93M formula diet and the diet mixed with 0.5% (w/w) freeze dried *B. L* TISTR for seven weeks. Blood was collected and immediately separated for plasma as explained in Materials and methods. Metabolic parameters were determined in plasma (Figure 2). Glucose and cholesterol levels were significantly lower in *B. L* TISTR fed mice than control mice. Furthermore, the insulin level was stimulated in *B. L* TISTR fed mice.

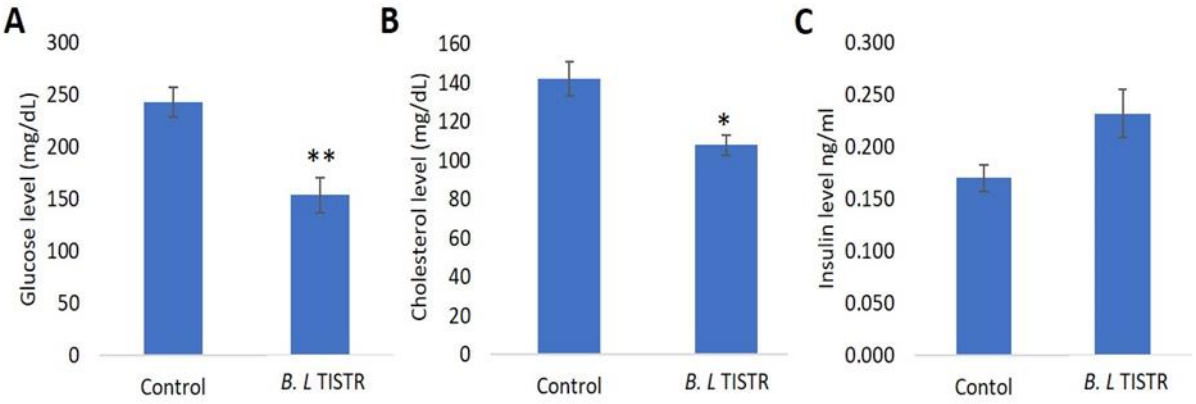

Figure 2 Blood metabolic parameters of mice in plasma; (A) glucose level, (B) cholesterol level and (C) insulin level. Statistical analyses were performed by means of ANOVA followed by Dunnett's test. Each value represents the mean ± SD (n = 6). $*p < 0.05$, $**p < 0.01$ versus the control group.

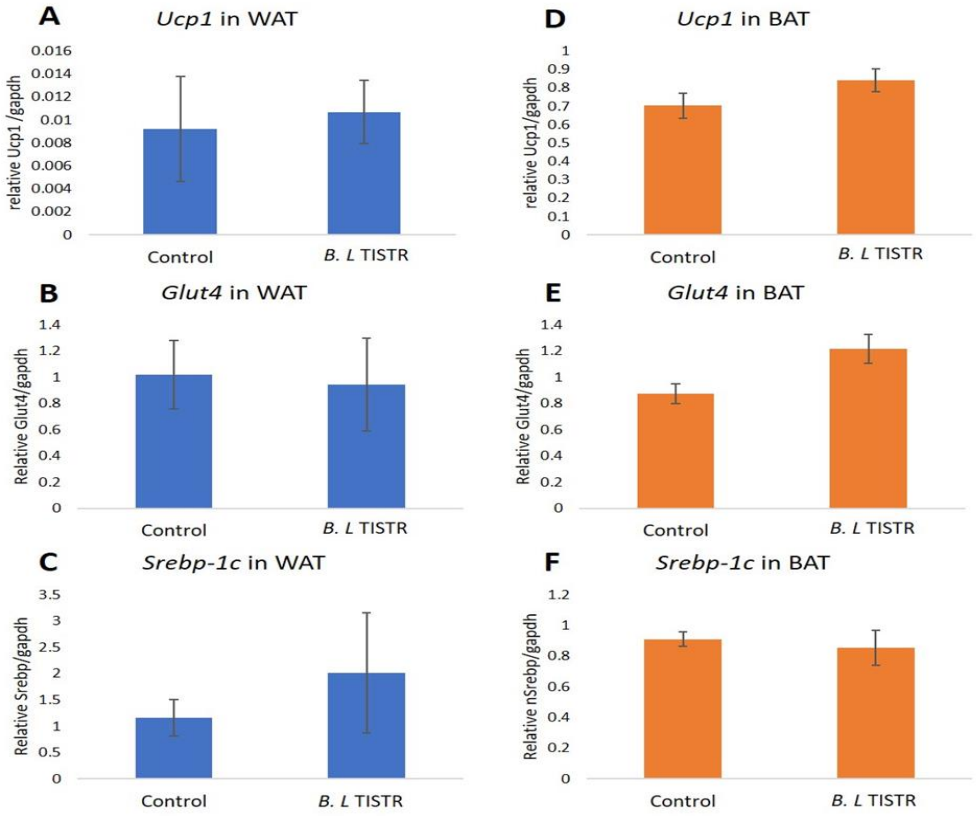

Figure 3 The effect of *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR) on metabolic genes expression that are related with insulin sensitivity. Expression of *Ucp1* (A, D), *Glut4* (B, E) and *Srebp-1c* (C, F) in white adipose tissue (WAT) and brown adipose tissue (BAT). Each value represents the mean ± SD (n = 6).

3.7. Determination of metabolic genes expression by real-time quantitative PCR

After free feeding of AIN93M formula diet and the diet mixed with 0.5 % (w/w) freeze dried *B. L* TISTR for seven weeks. White adipose tissue (WAT) and brown adipose tissue (BAT) were dissected. Total RNA from tissues and performed were extracted and used for qPCR of *Ucp1*, *Glut4* and *Srebp-1c*. The results showed that *Ucp1* and *Srebp-1c* were induced in white adipose tissue from *B. L* TISTR fed mice (Figure 3). While, *Ucp1* and *Glut4* were induced in brown adipose tissue from *B. L* TISTR fed mice. Liver also was dissected and performed qPCR for *Cyp7a*, *Ppara*, *G6pc* and *Gck*. The results showed that *Cyp7a*, *Ppara*, *G6pc* and *Gck* were stimulated in *B. L* TISTR fed mice (Figure 4). Moreover, we also examined qPCR for *Il10* and *Nos2* in Peyer's patches from intestine. The results showed that *Il10* was induced in *B. L* TISTR fed mice (Figure 5) whereas, *Nos2* decreased.

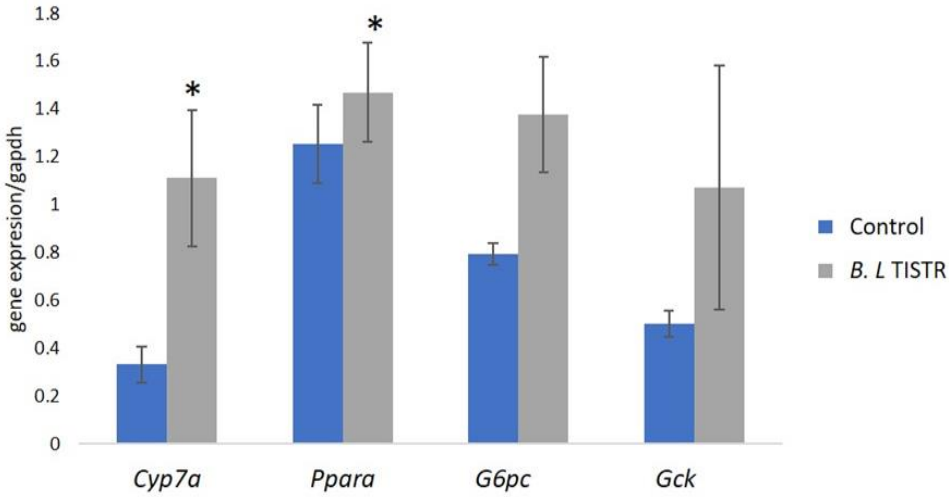

Figure 4 The effect of *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR) on metabolic genes expression (*Cyp7a*, *Ppara*, *G6pc* and *Gck*) in liver. Each value represents the mean ± SD (n = 6).

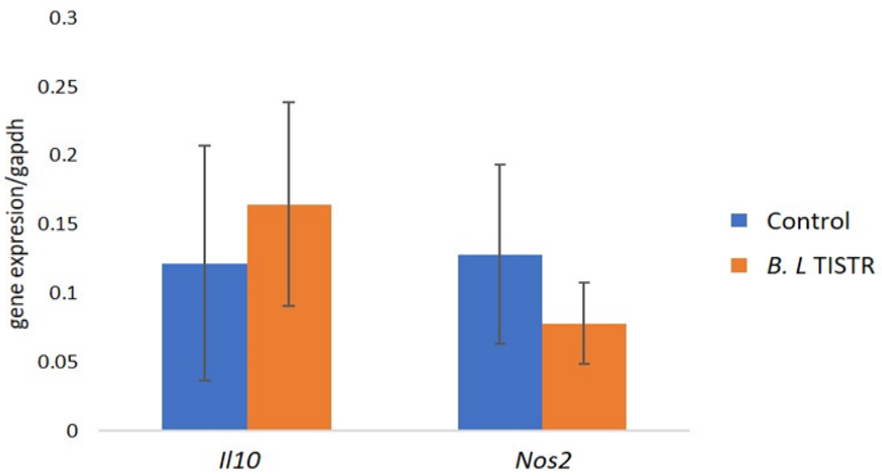

Figure 5 The effect of *Bifidobacterium animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR) on anti-inflammation activity (*Il10* and *Nos2*) in peyer's patches from intestine. Each value represents the mean ± SD (n = 6).

4. Discussion

Bifidobacteria is a dominant microbial group which is a common indigenous microbiota in the human intestinal tract [24]. It is a genus of lactic acid producing, Gram-positive, non-spore forming, non-motile, anaerobic bacteria. Bifidobacteria were first isolated from the feces of breast-fed infants in 1899 by Tissier [25] who observed that gut microbiota from healthy breast fed infants were dominated by rods with a bifid shape (bifidobacteria), even the word

"probiotic" was later coined 1960 reported by Ventura *et al*., [26]. Then, bifidobacteria had been isolated from a range of different ecological niches such as the oral cavity, sewage and the insect gut, the gastrointestinal tract of various mammals particularly human gastrointestinal tract (GIT) described and assessed by Turroni et al., [27]. Recently in 2016, a novel *Bifidobacterium* strain LMG 28769T was isolated from a household water kefir fermentation process by Laureys *et al*., [28]. In this study, we first reported a discovery of *B. animalis* subsp. *lactis* TISTR 2591 (*B. L* TISTR). It was isolated from meconium stools of 3-day old Thai infants. Regarding 16S rDNA analysis and whole genome sequencing technique, it was classified as *Bifidobacterium animalis* subsp. *lactis*. The strain "TISTR" indicates a discovery by the Thailand Institute of Scientific and Technological Research.

The probiotic property of *B. L* TISTR was assessed using various assays. Antibiotic resistance is a potential risk of probiotic application. Sensitivity to antibiotics is the most important factor in the safety evaluation of probiotics. We found out that *B. L* TISTR exhibited sensitivity to eight antibiotics including chloramphenicol, ampicillin, erythromycin, vancomycin, tetracycline, gentamicin, streptomycin and lindamycin indicated by MIC cut off values according to the European Food Safety Authority (EFSA, 2012) and the method of culture-dependent and culture-independent qualitative analysis of probiotic products claimed to contain bifidobacteria described by Masco *et al*., [29]. Two commercial probiotic strains, *B. lactis* and *L. reuteri* were used in this study and served as the reference probiotic strains. It was observed that *B. L* TISTR was more susceptible to ampicillin, chloramphenicol, vancomycin and erythromycin than *B. lactis* and *L. reuteri*. Moreover, *B. L* TISTR also displayed the greater antimicrobial activity than the two commercial probiotic strains when tested against 12 pathogenic bacteria of intestinal, oral and skin. Probiotics must be capable of surviving passage through the gastrointestinal tract (GIT) in order to have probiotic effects in the intestinal tract. Depending on the specific individual's diet, the pH of the human gastric environment varies from 1.5 to 3.0 and is usually around 3 reported by Beasley *et al*., [30]. Therefore, resistance to the gastric acid environment and bile tolerance are prerequisites for survival and function in the intestinal tract of probiotics. Regarding this issue, the in vitro tests were undertaken to determine desirable probiotic properties of *B. L* TISTR. Our results demonstrated that *B. L* TISTR exhibited a survival rate of 90.23% when exposed to pH 2 of gastric juice for 90 minutes. This finding indicates a good acid tolerance of *B. L* TISTR. It is a desirable probiotic property since it usually related to cross-resistance to some other stress factors, either those characteristic of the intestinal environment or typical of technological processes. Orally administered bifidobacteria firstly experience severe acidic conditions in the stomach where the pH is close to 2 and this strongly compromises bacteria viability. According to Kheadr *et al*., [31] and Knaust *et al*., [32], in general bifidobacteria tolerance to acid was reduced with the exception of *B. animalis*. Hence, our current study confirm their study. They also stated that acid tolerance response (ATR) was achieved through an assemblage of inducible molecular mechanisms. The pH resistant bifidobacteria strains with stable phenotype had been isolated after long exposure to acid pH. This stable phenotype was related to changes in the surface properties of the strains such as better adherence to mucin or pathogen displacement. However, at that time only an acid pH resistant *B. longum* strain had been performed.

Bifidobacteria are extensively used as probiotics by the food industry. Ruiz *et al*., [33] stated that different *Bifidobacterium* strains may show big differences in their tolerance to technological and physiological stresses. Therefore, the identification of strains showing greater tolerance among those presenting suitable probiotic properties is important for granting the efficacy of probiotic microorganisms included in food products. In order to reach the colon in a viable state, probiotics must cope with specific stress challenges throughout the GIT which the presence of bile in the upper parts of the small intestine. The main components of bile are bile acids which are produced and conjugated with the amino acids glycine or taurine in the liver to generate conjugated bile salts as documented by Hofmann [34]. Bile is stored in the gall bladder and flows from there to the duodenum during digestion, facilitating the solubilization and absorption of dietary fats. Under normal physiological conditions, our intestine holds the bile salt concentration to a range between 2% and 0.05% for shaping the microbial community profile found in our gut [35]. In our study, no fatal effect *B. L* TISTR when exposed to 0.3% bile salt for 4 hours. Based on results obtained from acid and bile tolerance tests, *B. L* TISTR seems to be suitable for oral administration. It can be used as a new bio-functional ingredient for food based probiotic products.

Among the main important characteristics of probiotic bacteria, adhesion to the intestinal mucosa is required. The adhesion to mucosal and epithelial surfaces is essential for successful immune modulation, competitive exclusion of pathogens as well as prevention of pathogen adhesion and colonization. Out study showed that *B. L* TISTR possessed in vitro adherence property to the human intestinal Caco-2 and HT-29 cell lines by 2.67 and 0.67% mucosal adhesion, respectively. The two commercial strains, *B. lactis* showed adhesion rate to Caco-2 by 0.35% and HT-29 by 0.29% whereas *L. reuteri* revealed adhesion rate by 0.75 and 0.89 % to Caco-2 and HT-29, respectively. This obtained data suggest that our potential probiotic *B. L* TISTR may have a potential capacity to colonise the gastrointestinal (GI) tract mucosa better than the commercial *B. lactis* and *L. reuteri* strains. This probiotic characteristic is important as it is involved in health benefit of the host.

Many researches have shown that probiotics play a role within various health areas. There is increasing evidence in favor of the claims of beneficial effects attributed to probiotics including improvement of intestinal health, enhancement of the immune response, reduction of serum cholesterol and cancer prevention. These health properties are strain specific and are impacted by the various mechanisms. Although, it has been well established that bifidobacteria confer positive health benefits to the human host, there is a clear lack of knowledge concerning the molecular mechanisms that explain these probiotic traits of *Bifidobacterium* [36]. In the present study, it was found that *B. L* TISTR could stimulate insulin level in plasma that was related with reducing glucose. As we expected, the glucose level in *B. L* TISTR fed mice decreased significantly. We also found that *G6pc* expression was induced in liver from *B. L* TISTR fed mice. Insulin can regulate hepatic gluconeogenesis via glucose-6-phosphatase (*G6pc*) that is involved in gluconeogenic control as previously reported by Barthel and Schmoll [37]. Therefore, we examined metabolic genes expression that are related with insulin sensitivity in white adipose tissue and brown adipose tissue. We found that *B. L* TISTR could induce insulin sensitivity in adipose tissue. However, *srebp-1c* was strongly induced in white adipose tissue. Sterol regulatory-element-binding protein-1c (*SREBP-1c*) is involved in the stimulation by insulin. In 1998, it was reported by Kim *et al*., [38] that insulin stimulates *SREBP-1c* gene transcription in adipose tissue. *SREBP-1c* is also involved in cholesterol and fatty acid metabolism according the study by Brown and Goldstein [39]. The mechanism of *SREBP-1c* to regulate cholesterol metabolism is by proteolysis of a membrane-bound transcription factor (HMG CoA reductase). It also regulates transcription of genes encoding many other enzymes in the cholesterol biosynthetic pathway. Thus, SREBPs coordinate the synthesis of the two major building blocks of membranes, fatty acids and cholesterol. Our study found that expression of *Gck* in liver was induced by *B. L* TISTR. It was reported that hepatic expression of *Gck* is up-regulated by the insulin signal via *SREBP-1c* [40].

We also found that cholesterol level in plasma *B. L* TISTR fed mice decreased significantly. Furthermore, the *Cyp7a* expression was induced significantly in liver from *B. L* TISTR fed mice. Massimi *et al*., [41] reported that cholesterol 7α-hydroxylase is major pathway for cholesterol catabolism that related to reducing cholesterol level. It is a rate-limiting enzyme in bile acid synthesis which is a major pathway for cholesterol catabolism. Our finding suggest that *B. L* TISTR induced insulin level which in turn reducing glucose and cholesterol level in plasma and liver. The nuclear receptor peroxisome proliferator-activated receptor alpha (*Ppara*) is a key transcriptional regulator of many genes involved in fatty acid β-oxidation. It plays a role in the catabolism of fatty acids in the liver reported by Manna [42]. Our result showed that expression of *Ppara* in liver from *B. L* TISTR fed mice significantly increased implying that induction of insulin by *B. L* TISTR could reduce fatty acid in liver.

Interleukin 10 (Il-10) also known as human cytokine synthesis inhibitory factor (CSIF), is an antiinflammatory cytokine. In humans, Il-10 is encoded by the *IL10* gene. *Il-10* was initially reported to suppress cytokine secretion. Sheil *et al*., [43] reported that interleukin 10 in knock-out (*Il-10* KO) mice could develop intestinal disease (chronic inflammation and enterocolitis) that associated with apparently dysregulated production of proinflammatory cytokines. Nitric oxide synthase 2 (*Nos2*) is a proinflammatory cytokine that affects innate immune system [44]. *Nos2* is important for protective immunity against cytomegalovirus (CMV). Therefore, we examined *Il10* and *Nos2* expression in Peyer`s patches from intestine. We found that *B. L* TISTR could induce *Il10* expression in Peyer`s patches from intestine. Moreover *Nos2* expression was found to decrease in *B. L* TISTR fed mice. These observations suggest that *B. L* TISTR may improve immune system and reduce inflammation activity that may provide for prevention of intestinal inflammation. In addition, future studies to evaluate anti-inflammatory and immune-stimulating activities of *B. L* TISTR will be undertaken by our group.

5. Conclusion

Bifidobacterium animalis subsp. *lactis* TISTR 2591 (*B. L* TISTR) is found to have potentially probiotic. It possesses pharmacological activities including blood glucose and cholesterol reduction, insulin stimulation and intestine antiinflammation. Results suggest that this probiotic strain can be a good candidate for food supplements and functional foods industries. Further studies can be undertaken to evaluate its other health beneficial properties as well as the clinical study to proof efficacy in volunteers.

Compliance with ethical standards

Acknowledgments

This study was financially supported by the Thai Royal Government, Ministry of Higher Education, Science, Research and Innovation (MHESI) through the Thailand Institute of Scientific and Technological Research (TISTR).

Disclosure of conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Statement of ethical approval

All procedures performed in studies involving animals were in accordance with the Guidelines for the Care and Use of Laboratory Animals at the National Institute of the National Institute of Advanced Industrial Science and Technology (AIST), Japan and the Animal Care and Use Committee at AIST approved the study protocol (Approval no. 2016-054F).

Author's contribution

Klungsupya Prapaipat (corresponding author) was responsible for the conception and design of the work and was involved in drafting and revising the manuscript. Saiki Papawee, Wannissorn Bhusita and Ruengsomwong Supatjaree were responsible in drafting and revising the manuscript. Muangman Thanchanok, Bamrungchue Nantana and Taengphan Weerasak participated in reading and commenting on previous versions of the manuscript. All authors read and approved the final manuscript.

References

[1] FAO/WHO - Food and Agriculture Organization of the United Nations/World Health Organization. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Report of a Joint FAO/WHO Expert Consultation. Cordoba.

[2] Lilly DM and Stillwell RH. (1965). Probiotics: Growth promoting factors produced by microorganisms. Science, 147, 747-748.

[3] Elizabeth CV and Susan L. (2010). Use of probiotics in gastrointestinal disorders: what to recommend? Therapeutic Advances in Gastroenterology, 3, 307-319.

[4] Yan F and Polk DB. (2011). Probiotics and immune health. Current Opinion in Gastroenterology, 27, 496-501.

[5] Reid G. (2008). Probiotic Lactobacilli for urogenital health in women. Journal Clinical Gastroenterology, 42, 234-236.

[6] De Alberti D, Russ R, Terruzzi F, Nobile V and Ouwehand AC. (2015). Archives of Gynecology and Obstetrics, 292, 861-867.

[7] Kumar M, Kumar A, Nagpal R, Mohania D, Behare P, Verma V, Kumar P, Poddar D, Aggarwal PK, Henry CJ, Jain S and Yadav H. (2010). Cancer-preventing attributes of probiotics: an update. International Journal Food Science and Nutrition, 61, 473-496.

[8] Figueroa-González I, Quijano G, Ramírez G and Cruz-Guerrero A. (2011). Probiotics and prebiotics - perspectives and challenges. Journal of the Science of Food and Agriculture, 91, 1341-1348.

[9] Holzapfel WH, Haberer P, Geisen R, Björkroth J and Schillinger U. (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. American Journal of Clinical Nutrition, 2, 365-373.

[10] Mercenier A, Lenoir-Wijnkoop I and Sanders ME. (2008). Physiological and functional properties of probiotics, Internation Diabetes Federation, 429, 2-6.

[11] Senok CA, Ismaeel YA and Botta GA. (2005). Probiotics: facts and myths. Clinical Microbiology and Infection, 11, 958-966.

[12] De Vrese M and Schrezenmeir J. (2008). Probiotics, prebiotics, and synbiotics. Advances in Biochemical Engineering/Biotechnology, 111, 1-66.

[13] Kaur PI, Chopra K and Saini A. (2002). Probiotics: potential pharmaceutical applications. European Journal of Pharmaceutical Sciences, 15, 1-9.

[14] Gomes A and Malcata FX. (1999). Bifidobacterium spp. and Lactobacillus acidophilus: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends in Food Science and Technology, 10, 139-157.

[15] Duffy LC, Zielezny MA, Riepenhoff-Talty M, Dryja D, Sayahtaheri-Altaie S, Griffiths E, Ruffin D, Barrett H, Rossman J and Ogra PL. (1993). Effectiveness of Bifidobacterium bifidum in experimentally induced MRV infection: dietary implications in formulas for newborns. Endocrine Regulation, 27, 223-239.

[16] Duffy LC, Zielezny MA, Riepenhoff-Talty M, Dryja D, Sayahtaheri-Altaie S, Griffiths E, Ruffin D, Barrett H, Rossman J and Ogra PL. (1994). Effectiveness of Bifidobacterium bifidum in mediating the clinical course of murine rotavirus diarrhea. Pediatric Research, 35, 690-695.

[17] Abou El-Soud NH, Said RN, Mosallam DS, Moniem Barakat NA and Sabry MA. (2015). Bifidobacterium lactis in Treatment of Children with Acute Diarrhea. A Randomized Double Blind Controlled Trial. Macedonian Journal of Medical Science, 3, 403-407.

[18] Pariyaporn I, Khawla S and Robinson RK. (2003). The potential therapeutic benefits of consuming health promoting fermented dairy products: a brief update. International Journal of Dairy Technology, 56, 203-210.

[19] Russell DA, Ross RP, Fitzgerald GF and Stanton C. (2011). Metabolic activities and probiotic potential of bifidobacteria. International Journal of Food Microbiology, 149, 88-105.

[20] Charteris WP, Kelly PM, Morelli L and Collins JK. (1998). Development and application of an *in vitro* methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. Journal of Applied Microbiology, 84, 759-768.

[21] Balouiri M, Sadiki M and Ibnsouda SK. (2016). Methods for in vitro evaluating antimicrobial activity: A Review Journal of Pharmaceutical Analysis, 6, 71-79.

[22] Saiki P, Kawano Y, Van Griensven LJLD and Miyazaki K. (2017). The anti-inflammatory effect of Agaricus brasiliensis is partly due to its linoleic acid content. Food and Function, 8, 4150-4158.

[23] Kanda Y. (2013). Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplantation, 48, 452-458.

[24] Reuter G. (2001). The Lactobacillus and Bifidobacterium microflora of the human intestine: Composition and succession. Current Issues in Intestinal Microbiology, 2, 43-53.

[25] Lilly DM and Stillwell RH. (1965). Probiotics: Growth promoting factors produced by microoganisms. Science, 147, 747-748.

[26] Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF and Chater KF. (2007). Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiology and Molecular Biology Reviews, 71, 495-548.

[27] Turroni F, Marchesi JR, Foroni E, Gueimonde M, Shanahan F, Margolles A, van Sinderen D and Ventura M. (2009). Microbiomic analysis of the bifidobacterial population in the human distal gut. ISME Journal, 3, 745-751.

[28] Laureys D, Cnockaert M, De Vuyst L and Vandamme P. (2016). Bifidobacterium aquikefiri sp. nov., isolated from water kefir. International Journal of Systematic and Evolutionary Microbiology, 66, 1281-1286.

[29] Masco L, Huys G, De Brandt E, Temmerman R and Swings J. (2005). Culture-dependent and culture-independent qualitative analysis of probiotic products claimed to contain bifidobacteria. International Journal of Food Microbiology, 102, 221-230.

[30] Beasley DE, Koltz AM, Lambert JE, Fierer N and Dunn RR. (2015). The evolution of stomach acidic and its relevance to the human microbiome. PLoS One, 10, 1-12.

[31] Kheadr E, Dabour N, Le Lay C, Lacroix C and Fliss I (2007). Antibiotic susceptibility profile of bifidobacteria as affected by oxgall, acid and hydrogen peroxide stress. Antimicrobial Agents and Chemotherapy, 51, 169-174.

[32] Knaust A, Weber MW, Hammerschmidt S, Bergmann S, Frosch M and Kurzai O. (2007). Cytosolic proteins contribute to surface plasminogen recruitment of Neisseria meningitides. Journal of Bacteriology, 189, 3246-3255.

[33] Ruiz L, Ruas-Madiedo P, Gueimonde M, de los Reyes-Gavila´n CG, Margolles A and Borja Sa´nchez B. (2011). How do bifidobacteria counteract environmental challenges? Mechanisms involved and physiological consequences. Genes and Nutrition, 6, 307-318.

[34] Hofmann AF. (1994). Bile acid. The liver: Biology and Pathobiology. First edition. Raven Press Ltd, New York, United States, 677-718.

[35] Islam KB, Fukiya S, Hagio M, Fujii N, Ishizuka S and Ooka T. (2011). Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology, 141, 1773-1781.

[36] Cronin M, Ventura M, Fitzgerald GF and Van Sinderen D. (2011). Progress in genomics, metabolism and biotechnology of bifidobacteria. International Journal of Food Microbiology, 149, 4-18.

[37] Barthel A and Schmoll D. (2003). Novel concepts in insulin regulation of hepatic gluconeogenesis. The American Journal of Physiology: Endocrinology and Metabolism, 285, 685-692.

[38] Kim JB, Sarraf P, Wright M, Yao KM, Mueller E, Solanes G, Lowell BB and Spiegelman B. (1998). Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. Journal of Clinical Investigation, 101, 1-9.

[39] Brown MS and Goldstein JL. (1997). The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell, 89, 331-340.

[40] Foretz M, Guichard C, Ferre P and Foufelle F. (1999). Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proceedings of the National Academy of Sciences of the United States of America, 96, 12737-12742.

[41] Massimi M, Lear SR, Huling SL, Jones AL and Erickson SK. (1998). Cholesterol 7α-hydroxylase (CYP7A): patterns of messenger RNA expression during rat liver development. Hepatology, 28, 1064-1072.

[42] Manna SK, Patterson AD, Yang Q, Krausz KW, Li H, Idle JR, Fornace AJ and Gonzalez FJ. (2010). Identification of noninvasive biomarkers for alcohol-induced liver disease using urinary metabolomics and the Ppara-nukk mouse. Journal of Proteome Research, 9, 4176-4188.

[43] Sheil B, MacSharry J, O'callaghan L, O'riordan A, Waters A, Morgan J, Collins JK, O'mahony and Shanahan F. (2006). Role of interleukin (IL-10) in probiotic-mediated immune modulation: an assessment in wild-type and IL-10 knock-out mice. Clinical and Experimental Immunology, 144, 273-280.

[44] Lowenstein CJ and Elizaveta P. (2004). iNOS (NOS2) at a glance. Journal of Cell Science, 117, 2865-2867.

How to cite this article

Klungsupya P, Saiki P, Wannissorn B, Ruengsomwong S, Muangman T, Bamrungchue N and Taengphan W. (2020). Probiotic property and biological activities of *Bifidobacterium animalis* subsp. lactis TISTR 2591 isolated from Thai population. World Journal of Advanced Research and Reviews, 5(3), 149-162.