TABLE OF CONTENTS

COMMITTEE OF
The 10th Asian Conference of Lactic Acid Bacteria (ACLAB-10)

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INTRODUCTION

ACLAB-10

The Asian Conference for Lactic Acid Bacteria (ACLAB) is a biennale event which is one of the activities facilitated by the Asian Federation of Societies for Lactic Acid Bacteria (AFSLAB). Each of the member countries of AFSLAB takes turn to host this event.

In 2019, it's a great pleasure for Indonesian Society for Lactid Acid Bacteria and Gut Microbiota (ISLAB-GM) to host the 10th Asian Conference on Lactic Acid Bacteria (ACLAB-10).

It is the aim of this conference to gather the advanced research information and application technologies of Lactic Acid Bacteria, Gut Microbiota, and Probiotic. The conference would serve as a platform for researchers from academia and industry in Asia to obtain and exchange information on scientific progress and applications.

PREFACE
Chairman of Organizing Committee

It is a great pleasure for the Indonesian Society for Lactic Acid Bacteria-Gut Microbiota (ISLAB-GM) to host The 10th Asian Conference for Lactic Acid Bacteria (ACLAB) in Yogyakarta, Indonesia. My warmest regards to all the speakers, honorable delegates, guests, and all participants.

The 10th ACLAB is held on 29 – 30 August 2019 at the Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta. The main aim of this conference is to bring together the advanced research and application technologies of Lactic Acid Bacteria, Gut Microbiota, and Probiotic. The conference would serve as a platform for researchers from academia, research institute, and industry in Asia to obtain and exchange information on scientific progress and applications.

This conference is hosting prominent speakers from twelve Asian countries, such as China, India, Indonesia, Iran, Japan, Korea, Malaysia, Mongolia, Phillippine, Singapore, Taiwan, and Thailand, and also a total of more than 200 participants from more than 15 countries, with the majority are from Asian countries. This conference is organized into five plenary lectures, four parallel sessions, oral and poster presentations. We wish that in this two-days event, we will have a great opportunity to share our works, extend network among students, scientists, and industries, and explore possibilities to work together in the future researches.

Aside from the conference, we would like to invite you to enjoy the cultural diversity Yogyakarta has to offer. We will depart to Prambanan temple on August 29 evening to have an exquisite dinner with Indonesian menu and later enjoy a remarkable performance of outdoor Ramayana

Ballet. On August 31 morning, we will have an excursion to Borobudur Temple, the largest Buddhist temple in the world and UNESCO World Heritage Site.

I would like to appreciate all the speakers, delegates, and participants for the tremendous efforts and time spent for the conference. We gratefully acknowledge the financial support from Yakult Indonesia. Also I would like to thank all of the sponsor, Prodia, Morinaga Jappan, Danone, Guangzhou Informa Yi Fan, Genetika Sciene, Kalbe, Fujicco, DIPA, BNI, 3M, and Fibercreme for their contribution in supporting this event. I would like to express my sincere gratitude to ISLAB-GM members, the Faculty of Agricultural Technology, and the members of organizing team for their contribution and helps - without them we would not have been able to organize this great meeting.

We wish the conference will have significant contribution in the field of Lactic Acid Bacteria, Gut Microbiota, and Probiotic both at Asian and worldwide level. I wish all of you lots of success during the conference and I hope you have a pleasant stay in Yogyakarta.

Tyas Utami

The 10th Asian Conference on
August, 28th - 31st 2019
Grha Sabha Pramana,
Universitas Gadjah Mada, Y

PREFACE
President of Asian Federation of Society for Lactic Acid Bacteria

Asian Federation of Society for Lactic Acid Bacteria (AFSLAB) was established in Tokyo, November 2002. A few months later, on 12 March 2003, a follow up was taken by the Indonesian members by establishing The Indonesian Society for Lactic Acid Bacteria (ISLAB) which has broadened its expertise into gut microbiota, and changed its name into The Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (ISLAB-GM) at the Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta. It is my greatest honor to be able to pioneer both of the prominent scientific organizations. Since AFSLAB was founded, we have successfully conducted biennial Asian Conference for Lactic Acid Bacteria (ACLAB) with the 10th ACLAB is hosted by ISLAB-GM in Indonesia in August 2019. This year will be the second time for Indonesia to host the same event after 3rd ACLAB in 2005 in Bali.

In the recent years, probiotic and gut microbiota have received tremendous attention from scientists around the world. AFSLAB has played important roles to nurture and develop a wide range of studies in lactic acid bacteria and gut microbiota, especially in Asian countries. Indigenous probiotic strains have been screened and selected for further use at industrial level. Probiotic is also believed to have beneficial effects to improve the status of several adverse health conditions such as stunting, diabetes, and obesity. All the aforementioned health conditions are receiving huge attention especially amidst the global efforts to improve human health condition in order to achieve the Sustainable Development Goals No.3 Good

Health and Well-Being. I strongly believe that our works are creating wide impacts for the society especially to improve the quality of human life.

On behalf of AFSLAB, I would like to appreciate all the speakers, delegates, and participants for their works in the field of lactic acid bacteria, and especially for their time and efforts to participate in this conference. I wish that the conference will be a great opportunity to bring together prominent scientists in Asia, along with students and industries and to explore possibilities to work together in the future.

I wish you good luck for the conference and I hope you have a wonderful time in Yogyakarta.

Endang S. Rahayu

PREFACE
Dean of Faculty of Agricultural Technology Universitas Gadjah Mada

Assalamualaikum wr wb.,

Greetings to everyone at 10th Asian Conference for Lactic Acid Bacteria (ACLAB-10)

First and foremost, on behalf of Faculty of Agricultural Technology Universitas Gadjah Mada, I would like to extend a warm welcome and my sincere gratitude to all invited speakers, industry professionals and participants from all around the world at the ACLAB-10 which is held in Faculty of Agricultural Technology Universitas Gadjah Mada.

It is a great honor to the Faculty to host the ACLAB-10 which provides important platform for networking and knowledge exchanges among researchers and industry professionals while educating the public on the role and application of lactic acid bacteria, probiotics and gut microbiota for food products and health. This conference is in line with our vision as Center of Excellent in the Agroindustry internationally. Food has been and will continue to be an integral part of human history. Application of lactic acid bacteria in food products and their role in human health will continue to deserve more attention. There is still much concern, curiosity and increasing interest among the researchers and public on the role of lactic acids bacteria, probiotics and gut microbiota on health and well-being. Therefore, this conference really provides the link and source of latest information for all parties concerned. The impressive list of presenters reflects the enthusiasm and participation in this conference.

Last but not least, I would like to congratulate and extend my appreciation to the organizing committee for hard work, great effort in coordinating this conference and for the sponsors for their everlasting support to this conference. We hope that all participants will benefits from this conference.

Thank you.
Wassalamualaikum wr. wb.

Eni Harmayani

PREFACE
President of The Indonesian Association of Food Technologists

Distinguished ladies and gentlemen,

On behalf of **The Indonesian Association of Food Technologists (IAFT) (*PATPI*)**, it is of my pleasure to welcome you all in this event - **The 10th Asian Conference on Lactic Acid Bacteria (ACLAB-10)**.

Conference, seminar, symposium, or other scientific forum is a common tradition held by professional organizations like **IAFT** and **AFSLAB** (the Asian Federation of Societies for Lactic Acid Bacteria). As the aim of this Conference is to gather the advanced research information and application technologies of Lactic Acid Bacteria, Gut Microbiota, and Probiotic, it is a very related concern with the field of food science and technology since most application of research of this area is utilized by the food industry. Food industry provides products that can contribute the public health. Therefore, the endeavor of AFSLAB members is in line with the mission of IAFT that is *Strengthening food science and technology in Indonesia for the prosperity of all.*

Regarding with international scientific forum, I would like to kindly inform you that IAFT this year will host **The 16th ASEAN Food Conference** (*The 16th AFC 2019*) that will be held in Bali, 15-18 October 2019. This conference is a biannual event held by **the Federation of the Institutes of Food Science and Technology in ASEAN (*FIFSTA*)**. I do hope that all participants here in this conference are also able to join and participate in the AFC 2019 Bali.

Again, may I take this opportunity to extend my warm welcome to you all, and to express gratitude to invited speakers, industry professionals, and all participants from over world. Congratulations, and a special appreciation is awarded to the Organizing Committee for hard work in arranging this enormous conference.

I wish you have a fruitful success in this Conference.

Thank you.

Umar Santoso

PREFACE
Chairperson of Indonesian Society for Microbiology

Distinguished ladies and gentlemen,

Let us pray to God the Almighty for His blessings bestowed on us so that we can be here to attend the 10th Asian Conference for Lactic Acid Bacteria (ACLAB) in Yogyakarta 28-30th August 2019. On behalf of the chairperson of Indonesian Society for Microbiology, I am delighted to convey my warmest welcome to Invited speakers, foreign delegates, speakers and participants.

Asian Conference for Lactic Acid Bacteria event is one of the activities facilitated by the Asian Federation of Societies for Lactic Acid Bacteria (AFSLAB). Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (ISLAB-GM) under Indonesian Society for Microbiology is a member of AFSLAB).

It is very pleasure for Indonesian Society for Lactic Acid Bacteria and Gut Microbiota to become host the 10th Asian Conference for Lactic Acid Bacteria in this year.

The ACLAB is an important biennial meeting. The meeting is one of the permanent agendas of AFSLAB, which held as a means to share their experiences and research results among researchers in Universities, research institute and industry.

I hope that all participants will be happy to have an opportunity to listen and discuss with the group of experts in the area of lactic acid bacteria and gut microbiome. I would like to congratulate the Organizing Committee for holding such an important conference. To the distinguished to speakers, participants, I hope you have a fruitful conference and enjoy stay in

Yogyakarta. I would like to express my high appreciation to Yakult Indonesia for supporting this conference.

To conclude my remarks on behalf of the chairperson of Indonesian Society for Microbiology, I hope the conference run effectively in achieving all theirs goals.

Siswa Setyahadi

PROGRAM ACLAB-10

1st Day (August 28th, 2019) Registration **2nd Day (August 29th, 2019)**		
07.30 - 08.00	Registration	
08.00 - 08.05	Opening	
08.05 - 08.15	Welcoming Dance "Tari Perjuangan"	
08.15 - 08.20	Welcome Remarks from Dr. Tyas Utami, M.Sc. as Chairman of ACLAB-10 Organizing Committee	
08.20- 08.30	Welcome Remarks from Prof. Dr. Ir. Endang S Rahayu, MS as President of AFSLAB	
08.30 - 08.35	Welcome Remarks from Prof. Dr. Ir. Eni Harmayani, M.Sc. as Dean Faculty of Agricultural Technology, Universitas Gadjah Mada	
Keynote Speakers		
08.35 – 09.05	Prof. Dr. Wilhelm Holzapfel	Are fermented foods still a promising source of beneficial LAB?
09.05 – 09.35	Prof. Dr. Min-Tze Liong	*Lactobacillus plantarum* DR7 and the Brain: Evidence and Mechanisms
09.35 – 10.05	Takuya Akiyama, Ph.D	The Count Counts: Applications of Yakult Intestinal Flora-Scan (YIF-SCAN) to Intestinal Microbiome Research
10.05 - 10.20	Coffee Break and Poster Session	
Plenary Lecture 1		
10.20 – 10.45	Prof. Ken Ichiro Suzuki	Occurrence of 10-hydroxy octadecanoic acid in the cellular fatty acids of *Lactobacillus acetotolerans*
10.45 – 11.10	Prof. Koichi Watanabe	From Phylogenomics to Taxonomy: The expected taxonomic changes in the genus *Lactobacillus*

11.10 – 11.35	Prof. Sung Shik Yoon	Restoration of Gut Microbiota by Administering Female Rat with LAB Culture Supernatants
11.35 – 12.00	Prof. Dr. Kenji Sonomoto	Lactic acid bacteria as a micro-refiner for sustainable green society
12.00 – 13.15	Lunch Break	
Plenary Lecture 2		
13.15 – 13.35	Prof. Jyoti Prakash Tamang	Bacterial community in naturally fermented milk products of India revealed by Culture-dependent and Culture-independent techniques
13.35 – 13.55	Prof. Dr. Hooi-Ling Foo	Lactic Acid Bacteria and The Way Forward
13.55 – 14.15	Prof. Francisco B. Elegado	Optimization Studies for Pediocin Production and Recovery from *Pediococcus acidilactici* 3G3
14.15 – 14.30	Coffee Break and Poster Session	
Plenary Lecture 3		
14.30 - 14.50	Dr. Jiro Nakayama	Features and signatures in Asian microbiome: Crisis or Adaptation?
14.50 - 15.10	Prof. Dr. Endang S Rahayu, MS	Gut Microbiota and Probiotics: Indonesian Perspective
15.10 - 15.30	Dr. G. Balakrish Nair	Distinct Gut Microbiome of healthy Indians Living in Urban and Rural Areas

15.30 - 15.50	Dr. Jin-zhong Xiao	*Bifidobacterium*
15.50 - 16.10	Prof. Yuan Kun Lee	Asian Microbiome Project Phase III: Infant microbiome
16.10 – 16.20	Closing	
16.30 - 21.30	Dinner and Ramayana Ballet Show at Prambanan Temple	

3rd Day (August 30th, 2019)		
07.30 - 08.00	Registration	
Room 1		
08.00 – 08.20	Dr. Yantyati Widyastuti	Role of lactic Acid Bacteria as Probiotics in the Rumen Fermentation
08.20 – 08.45	Prof. Seyed Shojaosadati	Intestinal adsorption of glucose, cholesterol and bile salt by simultaneous incorporation of edible microbiosorbent and intestinal bacteria
08.45 – 09.10	Dr. Massalin Nakphaichit	Alterations of gut microbiota associated with distinct allergic phenotypes: A longitudinal cohort study of Thai infants
09.10 – 09.35	Dr. Prakaash M Halami	Potentiality *Lactobacillus*

		plantarum group for probiotic functionality
Room 2		
08.00 – 08.20	Dr. Nanik Suhartatik	Microbial contamination of fresh juice sold in Surakarta
08.20 – 08.40	Prof. Dr. Teck Chwen Loh	Effect of Postbiotic and Inulin Supplements on Broiler Chickens
08.40 – 09.05	Prof. Ingrid Suryanti Surono	Understanding and Perception of Mothers on Probiotic and Prebiotics in Jabodetabek area
Room 3		
08.00 – 08.20	Prof. Dr. Lilis Nuraida	Cholesterol Lowering Effect of *Lactobacillus* Potential as Probiotic Isolated from Fermented Mustard
08.20 – 08.45	Prof. Dr. Demberel SH	Starter Culture Of Mongolian Traditional Fermented Milk Products And Their Application For Probiotic Strains
08.45 – 09.10	Dr. Rina Agustina. Ph.D	Maternal gut microbiota and

		Probiotic Supplementation as The Potential Interface of Gut-brain-axis in Promoting Fetal Brain Development and Child Cognition
09.10 – 09.35	I Nengah Sujaya. Ph.D.	Characterization Of *Weissella confusa* F213 As Probiotic
09.35 – 09.55	Jaka Widada. Ph.D.	Draft genome sequence of *Lactobacillus plantarum Mut-7* FNCC 250, a native Indonesian strain isolated from fermented dried cassava (gatot)
Room 4		
08.00 - 08.20	Dr. rer.nat. Agus Wijaya	Bile Salt Hydrolase Activities of Probiotic Lactic Acid Bacteria: Desireable or Undesireable?
08.20 – 08.45	Dr. Julie D Tan	Hazards in Traditional Fermented Foods
08.45 – 09.05	Dr. Tyas Utami, M.Sc.	Preparation of Indigenous Lactic Acid Bacteria Starter Cultures

		for Large Scale Production of Fermented Milk
09.05 – 10.15	Oral Presentation	
10.15 – 10.35	Coffee Break and Poster Session	
10.35 – 11. 20	Oral Presentation	
11.20 - 12.20	AFSLAB MEETING	
11.20 – 13.00	Break and Lunch	
13.00 – 15.00	Oral Presentation	
Plenary Lecture 4		
15.00 -15.25	Prof. Guo-Qing He	Lactic acid bacteria in HuangJiu and Its roles
15.25 – 15.50	Prof. Tadao Saito	Recent Strategy of Development of New Functional Yogurts Using Probiotics in Japan
15.50 – 16.15	Prof. Ming-Ju Chen	Subspecies Identification and Medium Optimization of Two Strains of Probiotic *Lactobacillus kefiranofaciens*
16.15 – 16.30	Coffee Break and Poster Session	
Plenary Lecture 5		
16.30 – 16.55	Prof. Yong-Ha Park	The Skin Gut Axis in Skin health and disease: A probiotic

		paradigm with therapeutic implications of Psoriasis and Atopic Dermatitis
16.55 – 17.20	Prof. Tsai Ying Chieh	Psychobiotics as Biotherapeutic Agents for Neurodegenerative Disorders
17.20 – 17.40	Dr. Neerja Hajela	The Growing Burden of Antibiotic Resistance – Can Probiotics help in reducing the crisis?
17.40 – 18.00	Closing Ceremony	
18.00 – 18.30	Break	
18.30 - 20.00	Dinner and Entertainment	

TECHNICAL SESSION PROGRAMME

Time	Note	Speaker	Title
Room 1			
09.35 - 09.55	A1	Saowanit	Prebiotic Effect of Plant Extracts on Growth of Probiotic Lactic Acid Bacteria and Fish
09.55 - 10.15	A2	Rio Jati Kusuma	Divergent Effect Of Rice Bran And Fermented Rice Bran On Cecal Short Chain Fatty Acid And Lactic Acid Bacteria Of Colorectal Cancer Model Of Mice
10.35 – 10.55	A3	Chen, Ying-Chen	Investigation on The Responses of *Lactobacillus mali* APS1 to Environmental Stresses and Its Survival After Freeze-Drying
10.55 – 11.15	A4	Yu-Ting, Hsu	Evaluating The Correlation Between Microbiome And Metabolome Associated with The Mastitis in Holstein Dairy Cows
13.00 – 13.20	A5	Li, Kai-Yi	Development of Fermented Milk with *Lactobacillus paracasei* PS23 and Evaluation of Its Anti-colitis Function
13.20 – 13.40	A6	Nurulfiza Mat Isa	Isolation, Characterisation and Anti-Breast Cancer Effects of Potential

			Probiotic Bacteria from Human Breast Milk
13.40 – 14.00	A7	Marilen Parungao Balolong	Cytotoxic Activity of The Biofunctional Probiotic Strains *Lactobacillus plantarum* Bs25 and *Pediococcus acidilactici* S3 Against Colorectal Cancer Cells (Hct 116)
14.00 – 14.20	A8	Yoyok Budi Pramono	Utilization of Lesser Yam (*Dioscorea esculenta L.*) Flour as Prebiotic in Yogurt to Total Lactic Acid Bacteria (LAB), Sugar Reduction, and Organoleptic Properties
Room 2			
09.05 – 09.25	B1	Adelene Song AI Lian	*Lactococcus lactis* Producing Phage Lysins as Potential Enzybiotics Against Methicilin Resistant *Staphylococcus aureus*
09.25 – 09.45	B2	Karseno	The Effect of Dates Addition and Fermentation Time on Quality Characteristic of Coconut Water – Dates Probiotic Drink
09.45 – 10.05	B3	Gerry Michael Donad Harindah	Isolation And Identification Lactic Acid Bacteria From Gedi Leaves (*Albemoschus manihot L.*)
10.05 – 10.25	B5	Usman Pato	Antimicrobial activity of lactic acid bacteria strains isolated from

			dadih against *Listeria monocytogenes*
10.40 – 11.00	B6	Artitaya Buatong	Investigation of Antioxidant Activity and Cholesterol Reducing Ability from Lactic Acid Bacteria and *Bacillus spp*. Isolated from Foods and Fish Samples
11.00 – 11.20	B7	Ryan Haryo Setyawan	Edible mushroom potency to alleviate stunting through gut microbiota modulation : a review
13.00 – 13.20	B8	Leslie Michelle M. Dalmacio	Prebiotic Effects Of Philippine Medicinal Plants On Mice Gut Microbiome Provide Support For Their Health Benefits
Room 3			
09.55 - 10.15	C1	Laksmi Hartajanie	Effect of Administration Fermented Bitter Melon Juice on Lipid Profile of Diabetic Sprague Dawley Rats
10.35 – 10.55	C2	Lindayani	Potential Of Ampel Bamboo Shoots (*Bambusa vulgaris*) Picle "*Lactobacillus fermentum LLB3*" and "*Lactobacillus pentosus LLA18*" As a Starter For Mozzarella Cheese And Beverage
10.55 – 11.15	C3	Ratchanu Meidong	Screening and Partial Characterization of Bacteriocin Produced by

			Lactic Acid Bacteria from Traditional Thai Fermented Food
13.00 – 13.20	C4	Priyanka Parhi	Effect of Fructooligosaccharides On The Growth and Survival of *Lactobacillus plantarum* in Model Sugar Systems
13.20 – 13.40	C5	Vita Meylani	Microbial Diversity in Traditional Kefir using culture-independent methods
13.40 – 14.00	C6	MD Rakhimuzzaman	Establishment of an Efficient Method of Ornithine and Citrulline High-production by a Plant-derived Lactic Acid Bacterium, *Weissella confusa* K-28
14.00 – 14.20	C7	Vaibhao Kisanrao Lule	Zinc Enrichment of *Lactobacillus spp*. and Assessment of its Bioavailability in Albino Wistar Rats: A Novel Biological Approach with Improved Bioavailability
14.20 – 14.40	C8	Thanawat Namrak	Genome sequencing through reconstruction of genome-scale metabolic network of *Lactobacillus reuteri KUB-AC5*
14.40 – 15.00	C9	Siti Nur Husnul Yusmiati	The Effect of Lactobacillus casei Strain Shirota on Hemoglobin Level among Female Adolescents

Room 4			
09.05 – 09.25		Saiful Fazulul Haq	Importance of Yeast Derived Nutrients for Industrial Production of Lactic Acid Bacteria and Probiotics
09.25 – 09.45	D1	Amelia Juwana	Identification Of Probiotic Potential Lactobacillus From Mandai Using Molecular Technique
09.45 – 10.05	D2	Phatthanaphong Therdatatha	Difference of Gut Microbial Community in Indonesian Obese, Type 2 Diabetic, and Healthy Groups
10.05 – 10.25	D3	Yuri Lee	Development And Validation Of A Novel In Vitro Probiotic Screening System
10.40 – 11.00	D4	Arellano Ayala Karina	Rehydration improves probiotic properties of lyophilized *Lactobacillus plantarum*
11.00 – 11.20	D5	Dyah Fitri Kusharyati	Isolation of Bifidobacterium from Infant's Feces on Its Antimicrobial Activity
13.00 – 13.20	D6	Anang Mohamad Legowo	Effect Of D-Tagatose As Substrate On The *Lactobacillus bulgaricus* Existed Medium
13.20 – 13.40	D7	Hanies Ambarsari	Influence of Lactic Acid Bacteria in Commercial Probiotic on the

			Nitrification Process by Bacteria from Catfish Pond Sediment
13.40 – 14.00	D8	Ngatirah	Effect Of Iles-Iles (Amorphopallus Oncophyllus) Sinbiotic Effervescent Tablet To Decrease In Blood Sugar Levels In Hyperglicemic White Mice (Rattus Norvegicus)
14.00 – 14.20	D9	Virginia P. Obsioma	*Lactobacillus paracasei* C2i12 Treatment Of Meat From Old Philippine Native Cows
14.20 – 14.40	D10	Somchai Jaikhan	Potent of lactic acid bacteria isolated from Thai fermented meat for protease production

LIST OF POSTER

POSTER PRESENTER			
1	Adi Yulandi	Contribution of Lactic Acid Bacteria to Vitamin B12 Content in Indonesian Tempeh	**PP-1**
2	Aditya R. Suharto	Bacterial Population Dinamics Of Natural Fermentation Of Sumbawa Mare's Milk Using Metagenomic Approach And Physicochemical Aspects	**PP-2**
3	Agussalim Matti	Isolation of Proteolytic Lactic Acid Bacteria from Indigenous Chao Product	**PP-3**
4	Ahmad Faizal Fajar Sunama	Antimicrobial Activity of Lactic Acid Bacteria Isolated From Ronto, a Traditional Fermented Shrimp Paste from South Kalimantan	**PP-4**
5	Ahmad Junaidi	Production of Synbiotic Drinks from Purple Sweet Potato *(Ipomoea batatas)* with Probiotics *Lactobacillus plantarum B1765*	**PP-5**
6	Ahmad Mustangin	The Effects of Consumption of Indigenous Probiotic Powder Containing *Lactobacillus plantarum Dad-13* on The Body Mass Index and The Population of *Prevotella*, *Bacteroides fragilis* and *Clostridium coccoides* on Malnourished Children of Belanting Elementary School, East Lombok	**PP-6**
7	Ambar Rukmini	The Effect of Celery Yogurt Intervention on The Lipid Profile of Wistar Rat Fed High Oxidized Fat	**PP-7**

8	Aninditya Ratnaningtyas	The Effects of Consuming Capsule Containing *Lactococcus lactis subsp. cremoris FC* to Overcome Constipation of Indonesian Women	**PP-8**
9	Ardiansyah	Novel Function of Dual Fermented Rice Bran to Improve Metabolic-Related Diseases in Shrsp	**PP-9**
10	Ari Surya Sukarno	Detection of Putative Genes Encoding Bile Salt Hydrolase (Bsh) in Probiotic Strains Isolated from Indonesia Local Cultures	**PP-10**
11	Atipat Yasiri	Isolation of Probiotic Lactic Acid Bacteria with Bile Salt Hydrolase and Uricase Activity from Fermented Spider Plant	**PP-11**
12	Baiq Rani Dewi Wulandari	Antioxidant Activities and Viability of Lactic Acid Bacteria in Yogurt Made from Buffalo Milk with Addition of Blewah (*Cucumis melo L var. reticulatus Naudin*) Juice	**PP-12**
13	Catharina Tri Widyastuti	Fermentation Effectivity of Milk Supplemented with *Ficus hispida Linn.F.* Leaves Extract by *Lactococcus lactis subsp. lactis*	**PP-13**
14	Chang, Hsiao-Tung	Effect of Probiotic Additive SYNSEATM FeedAd on Growth Performance, Antimicrobial Ability and Gut Morphology of White Shrimp in Field Trial	**PP-14**
15	Dugersuren Jamiyan	Results of Some Molecular Biological Studies on Local Strains Lb *Plantarum LAB3c, LAB 19x, LAB58a AND Lb. casei LAB 26a, 53a*	**PP-15**

16	Eko Farida	Down-Regulation Of Gluconeogenic Gene Expression Indigenous Lactobacillus rhamnosus	**PP-16**
17	Erdene Bayar	Anti-bacterial peptides isolated from *Bifidobacterium bifidum* in young ovis aries	**PP-17**
18	Evi Triana	The Effect of Mangosteen Extract on Viability of *Lactobacillus plantarum* Mar8 In Black Cincau Ready to Drink (Rtd) Beverage	**PP-18**
19	Goh Chee Yong	Effects of scGOS/lcFOS (9:1) + 2'-FL Combination on Infant Gut Microbiota Composition and Metabolic Activities Using In Vitro SHIME®	**PP-19**
20	Hazel Alena D. Tan	Linamarase-Producing Lactic Acid Bacteria For Cassava (*Manihot esculenta Crantz*) Sourdough Fermentation	**PP-20**
21	Helen Joan Lawalata	Lactic Acid Bacteria From Langsat Fruit In Manado	**PP-21**
22	Ida Bagus Agung Yogeswara	Isolation, Screening and Identification of Γ-Aminobutyric Acid Producing Lactic Acid Bacteria from Indonesian Fermented Foods	**PP-22**
23	In-Chan Hwang	Construction of Expression Vector with a Bile-responsive Promoter in *Lactobacillus plantarum*	**PP-23**
24	Intan Niken	Viability Local Probiotics on Yogurt with Supplementation of Purple Sweet Potatoes to Prevention of Diarrhea in Experimental Rats	**PP-24**
25	Ismi Hidaya	Development Of Gelato Synbiotic From Soygurt And Lesser Yam	**PP-25**

		(*Dioscorea Esculenta L.*). Review Of Chemical, Microbiology, And Organoleptic Quality	
26	Ji Hoon-Song	Optimization of Culture Conditions for the Growth of *Bacillus coagulans TM3*	**PP-26**
27	Jirawan Apiraksakorn	The Identification of Lactic Acid Bacteria Isolated from Thai Fermented Meat Using 16S rDNA Technique	**PP-27**
28	Johanna A. Bangoy	Microbiological, Physico-Chemical and Organoleptic Assessment of a Carrot-Milk Drink Formulation Using *Lactococcus lactis Sc1, Lactobacillus plantarum Bs and Pediococcus acidilactici 3g3* as Potential Probiotic Starter Cultures	**PP-28**
29	Ju Kyoung Oh	Synergistic Effect Of *Lactobacillus gasseri* and Cudrania tricuspidata on Anti-Obesity in High-Fat Diet-Fed Mice	**PP-29**
30	June Su Yin Low	Simulator of the Human Intestinal Microbial Ecosystem (SHIME®):Effect of Prebiotic Mix Combinations on Gut Microbiota Composition and Short-Chain Fatty Acids (SCFAs)	**PP-30**
31	Kazuya Toda	*Bifidobacterium breve B-3* Promoted Oxidative Muscle Fiber Composition thorough Activation of AMPK-PGC1α-Mitochondrial Biogenesis Pathway	**PP-31**
32	Komang Ayu Nocianitri	Improving Perfomance And Health Promoting Effects Of Fermented Milk Using Sweet Potato Powder	**PP-32**

33	Mariyatun	Effect of Consumption Indigenous Probiotic Powder of *Lactobacillus plantarum* DAD-13 in Maintaining Health in Obese in Yogyakarta: Preliminary Study	**PP-33**
34	Lily Arsanti Lestari	Sensory Evaluation and Microbiological Properties of Probiotic Yogurt Made with Different Type of Probiotic Cultures Starter	**PP-34**
35	Ma Xia	Effect of Regular Consumption of the Probiotic *Lactobacillus paracasei* strain ET-22 on the Intestinal Microbiota of Mice	**PP-35**
36	Merkuria Karyantina	Aplication *Pediococcus sp (Pediococcus halophillus* and *Pediococcus acidilactici*) on Lactic Acid Bacteria of Jambal Roti (Fermented Fish) With NaCl Variation	**PP-36**
37	Musaalbakri Abdul Manan	Bioprocessing Strategies to Enhance and Develop The Functionality and Added Value on Generic Fermented Rice Nutrient	**PP-37**
38	Nancy Eka Putri Manurung	Effect of Consumption Indigenous Probiotic Powder of *Lactobacillus Plantarum Dad-13* of Students SMP N 1 Pangururan on The Population of Gut Microbiota	**PP-38**
39	Neysa Fitri	Isolation and Characterization of Lactic Acid Bacteria from Legume Soaking Water of Tempe Productions	**PP-39**
40	Ni Nengah Dwi Fatmawati	Adhesion Activity and Antimicrobial Resistance Profile of *Weissela confusa* Probiotics Strain	**PP-40**

41	Ni Wayan Eka Putri Gayatri Kastawa	Phenotype and Genotypes Antibiotic Resistant Profiles of *Lactobacillus* Local Strains	**PP-41**
42	Ni Wayan Nursini	Antioxidant Activity, Fiber, Acidity (Ph) and Population of Lactic Acid Bacteria (Bal) in Green Juice as Functional Beverages	**PP-42**
43	Ninik Rustanti	The Effect of Prebiotic Ginger Yogurt on Fasting Blood Glucose, HbA1c, Insulin, Blood Pressure and Malondialdehyde in Prediabetes Women	**PP-43**
44	Noerman Yusuf Pratama Putra	Potential of *Lactobacillus plantarum B1765* in Fermentation of Soybean Extract as Antihyperglycemic Alternative Therapy	**PP-44**
45	Nova Wahyu Pratiwi	Isolation and Characterization of Lactic Acid Bacteria from Riau Traditional Tempoyak	**PP-45**
46	Yuliana Tandi Rubak	Angiotensin Converting Enzym Inhibitory (Ace-I) In Fermented Soy Milk by Indigenous Lactic Acid Bacteria	**PP-46**
47	Miftha Fachruri	Growth of Lactic Acid Bacteria and Acid Production in Jack Bean Milk (*Canavalia ensiformis* (L.) DC.) Added with Sucrose and Skim-Milk	**PP-47**
48	Pratama Nur Hasan	The Effect of Consumption Indigenous Probiotic Powder Containing *Lactobacillus plantarum Dad-13* of Students SMPN 1 Pangururan, Samosir on Weight, Body Mass Index and The Population Of Gut Microbiota	**PP-48**

		Prevotella, Bacteroides fragilis and *Clostridium coccoides*	
49	Prima Retno Wikandari	Antioxidant Properties of Single Garlic (*Allium sativum*) Picle	**PP-49**
50	Qori Emilia	Viability of *Lactobacillus plantarum* in Cocoa Powder and Dark Chocolate Compound	**PP-50**
51	Rafli Zulfa Kamil	Microencapsulation of Indigenous Probiotic *Lactobacillus plantarum DAD 13*: Strain Dependent Effect and its Antibacterial Property	**PP-51**
52	Ratu Safitri	Evaluation of The Probiotic Properties of *Lactobacillus paracasei* and *L. curvatus* Isolated from Bovine Colostrum and Encapsulated in Dry Preparations	**PP-52**
53	Ridwan Rizkyanto	Effect of Consumption of Indigenous Probiotic powder *L. plantarum Dad-13* on Lipid Profile and Fecal Microbiota of Overweight Subjects Living in Yogyakarta, Indonesia	**PP-53**
54	Roslina Binti Jawan	Acid Bacteria Isolated From Raw Milks and Dairy Products of Sabah (Northern Borneo), Malaysia	**PP-54**
55	Siam Popluechai	Gut Microbiota of Thai Colorectal Cancer Patients	**PP-55**
56	Siti Nur Purwandhani	Antimicrobial Activity Of *Lactobacillus plantarum G-3* Isolated From Dadih Against Pathogenic Bacteria	**PP-56**
57	Sri Sumarsih	The Effect of Different Methods of Giving Probiotics *Lactobacillus Sp*. on Broilers Performance in Starter Period	**PP-57**

58	Stella Magdalena	Isolation and Evaluation of Probiotic Potential of Lactic Acid Bacteria from Oncom	**PP-58**
59	Suharman	The Effects of Consumption of Indigenous Probiotic Powder containing *Lactobacillus plantarum Dad-13* in Malnourished Children of Belanting Elementary School, East Lombok on The Population of Gut Microbiota *L. plantarum, Bifidobacterium, Enterobacteriaceae* and Short-Chain Fatty acids	**PP-59**
60	Sun Ting	Regulatory Effect of the Putative Probiotic *Bifidobacterium lactis* Strain BL-99 on the Intestinal Flora of Mice	**PP-60**
61	Surat Vangpikul	Effects of Probiotics *Lactobacillus* on Japanese quails production	**PP-61**
62	Susana Ristiarini	Colostrum Yogurt: Antimicrobial Activity and The Effect on Rat Fecal Microbes	**PP-62**
63	Svetoslav Dimitrov Todorov	Can *Enterococcus* hirae be a Nisin Producer?	**PP-63**
64	Ting Y. Lee	Effects of Probiotic in Pelleted Feed on Growth Performance of Broilers	**PP-64**
65	Titiek Farianti Djaafar	Microbiology, Chemical and Sensory Characteristics of Cocoa Powder: The Effect of *Lactobacillus plantarum Hl 15* as Culture Starter and Fermentation Box Variation	**PP-65**
66	Tri Marwati	Viability and Antifungal Activity of *Lactobacillus plantarum Hl-15* Oven Dried Culture During Storage	**PP-66**

67	Vichai Leelavatcharamas	Isolation of Lactic Acid Bacteria Capable of Cancer Cell Growth Inhibition from Thai Local Fermented Foods	**PP-67**
68	Wahyu Hidayati	Molecular Detection of Antibiotics Resistant-Encoded Genes of *Lactobacillus rhamnosus SMM* Strains	**PP-68**
69	Wilawan Sintuprapa	Antagonistic Effects of Fermented Soybean Meal as A By-Product on The Growth of *Streptococcus agalactiae* Bovine Mastitis Pathogens	**PP-69**
70	Wisnu Adi Yulianto	The Level of Aerobics in The Production of Black Glutinous Rice Tape and Its Potential as a Probiotic Food	**PP-70**
71	Yeanly Wuena	Optimization Of Exopolysaccharide Production By *Lactobacillus Casei Al.15*	**PP-71**
72	Yogiara	Metagenomics Study Revealed that Oncom, a Peanut Press-cake Fermentation Food, is a Promising Source of Lactic Acid Bacteria	**PP-72**
73	Zhao Wen	In-vitro and in-vivo Evaluation of the Probiotic Potential of *Lactobacillus paracasei* strain K56 for Modulating Gastrointestinal Health	**PP-73**
74	Ida Ayu Kade Ratna Sukmadewi	Hemolysis Activity of *Lactobacillus* Local Strains, The Probiotics Candidates	**PP-74**
75	Hisakazu Iino	Prebiotic Effect of 2 g of Lactulose: A Randomized, Double Blind, Placebo-Controlled Crossover Study	**PP-75**

76	Delima Citra Dewi	Organoleptic Assessment, Cholesterol Level and Total Colony of Lactobacillus Acidophilus In Piskebal (Pisang Kepok Bakteri Asam Laktat)	**PP-76**
77	Rita Khairina	Fermented Food Diversity in South Kalimantan	**PP-77**
78	Pierangeli G. Vital	Assessment of Microorganisms Isolated from Commercially-Available Probiotic Products Suitable for Children Being Marketed In The Philippines	**PP-78**
79	Gaku Harata	*Lactobacillus rhamnosus* GG Might Influences Gut Microbiota of Healthy Japanese Subjects with a Possible Sex-Dependent Way	**PP-79**
80	Agustina Intan Niken Tari	Viability Local Probiotics on Yogurt with Supplementation of Purple Sweet Potatoes to Prevention of Diarrhea in Experimental Rats	**PP-80**
81	Nita Maria Rosiana	Development of Kefir with Adding of Dragon Fruit Peel Extract as a High Antioxidant Drink	**PP-81**

ABSTRACTS OF INVITED SPEAKERS

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Potential Application of Probiotics for Acrylamide Mitigation in Food Products

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ABSTRACT

Acrylamide is a process-induced food toxicant which is formed during heating of food products rich in carbohydrates at high temperature and low moisture conditions. Its formation is affected by several factors such as temperature, time, type and level of reducing sugars, pH and moisture content. Studies have revealed that acrylamide causes DNA damage, neurotoxicity, genetic toxicity, reproductive toxicity and carcinogenicity. Therefore, efficient approaches should be applied to reduce acrylamide level in food products. Some of these methods include reduction of precursors in raw materials, changing the process parameters such as temperature, pH and addition of amino acid and salts and post processing approaches like chromatography, evaporation and polymerization. However, most of the abovementioned approaches are not practical, requires costly equipment and cause nutrition loss and decline of sensory properties. In this regard, using specific strains of probiotics has been explored. Several studies demonstrated probiotics such as *Lactobacillus plantarum, Lactobacillus casei and Lactobacillus reuteri* can mitigate acrylamide level in foods. The mechanism responsible for this activity is associated with the presence of cell wall peptidoglycan and protein which binds to acrylamide. It has been pointed out that hydroxyl groups of either glucose or glycerol in teichoic acids might be involved in interactions with carbonyl oxygen atoms of acrylamide. Another possible mechanism is production of the enzyme asparaginase by probiotic strains which converts L-asparagine to L-aspartic acid and ammonia and prevents acrylamide formation. Considering the findings in different studies, it can be concluded that selection of suitable probiotic strains can play an important role in reduction of acrylamide in foods and it they can be used in development of new functional foods.

Keywords: Acrylamide, Binding, Mitigation, Probiotics.

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Yogyakarta, August 29-31, 2019

Starter Culture of Mongolian Traditional Fermented Milk Products and Their Application for Probiotic Strains

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ABSTRACT

The tradition of making fermented dairy products and their starters , which is inherited to this day through the Mongolian family - herdsmen's for many centuries, is one of the most unique and rich resources of lactic acid bacteria. We are a number s bacterial strains isolated from fermented mare's milk "Airag" and "Tarag"(similar the yogurt) in Mongolia, were identified phylogenetically based on rDNA and functional genes sequences. We confirmed that Airag and Tarag had unique LAB compositions, that is, *L. helveticus, L. kefiranofaciens* were the predominant in Airag, whereas *L. delbrueckii subsp. bulgaricus, L. helveticus* and *S. thermophilus* were the predominant in Tarag. These findings suggested strongly that the differences among the animal species from which the milk was sourced, were the most important factors influencing the diversity of microbial composition. The information obtained from our research study of Airag and Tarag will be useful to find for probiotic strains with distinctive features of Mongolia.

With the aim of the most effective strains suitable for probiotics, we have isolated 115 primary cultures from traditional fermented dairy products. Of these, selected a number of LAB strains as a result of a laboratory biomedical examination, such as cultural-biochemical, antigonistic, resistant, and physiological properties. In this case, as an example of these studies, we will present the probiotic properties of *L.helveticus LBMA 44c* and *L.bulgaricus subsp LBMA 65b* isolated from the fermented mare' s milk "Airag". In addition to their cultural, physiological and biochemical qualities, they possess antigonistic properties against enteropathogenic and resistant to the action of antibiotics, alkalis, and acid, and also proliferate well in low pH conditions, which we considered one of the important properties of probiotic LAB strains.

Keywords: lactic acid bacteria

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Gut Microbiota and Probiotics: Indonesian Perspective

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ABSTRACT

Recent studies has associated gut microbiota with various diseases other than intestinal disease. There has been rapid growth in the study of gut microbiota. Numerous factors influence gut microbiota, such as age, diet, geographical area. Gut microbiota of Indonesian subjects represented by young and elderly Javanese and Balinese tribes have been studied by fecal samples analysis. Bacteria population in feces of younger subjects was higher than that of elderly groups, with a total of approximately 10.0-10.6 $\log_{10}$ bacterial cells/g feces. The most abundant bacteria were *Clostridium*, followed by *Prevotella, Atopobium, Bifidobacterium* and *Bacteroides*. In elderly subjects, an increase in *Enterobacteriaceae*, coliform and *Escherichia coli* was found. Furthermore, among genus *Lactobacillus, L. plantarum* subgroup, is one of the most dominant subgroup and has the highest prevalence (>85%), followed by *L. gasseri*, and *L. reuteri*, while *L. ruminis* despite having a high population, had low prevalence (< 80%). With regard to *L. casei* subgroup, the number and prevalence were low. Composition of gut microbiota can be used to determine which treatment is suitable and effective to improve gut health status. Genus *Lactobacillus* is one of LAB with a great number of potentials as probiotic. Several new strains of probiotic

had been isolated from Indonesian fermented food, and one of them is identified as *Lactobacillus plantarum*. These strains include *L. plantarum* Mut-7 isolated from *gatot* (fermented cassava) and *L. plantarum* Dad-13 isolated from *dadih* (spontaneous buffalo fermented milk). Furthermore, health benefits of indigenous probiotic were tested in several regions in Indonesia, namely Yogyakarta, Samosir and Lombok. The study showed that there is a change of gut microbiota composition after two months of probiotic consumption in undernourished school-aged children in Lombok. Additionally, an improvement in body mass index was also observed. However, there is no significant effect after one-month probiotic consumption in healthy school-age children in Yogyakarta. Interestingly, the prevalence of *L. plantarum* is quite high in the gut microbiota composition of Indonesian, which may be correlated with the dominance of *L. plantarum* found in traditional fermented food from Indonesia. Therefore, it can be expected that probiotic from species *L. plantarum* is suitable for Indonesian people. For further research, the effects of indigenous probiotic will be tested to alleviate metabolic disorders such as diabetes and obesity.

Keywords: Gut Microbiota, Lactobacillus plantarum, indigenous probiotic, Indonesian fermented food

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Optimization Studies for Pediocin Production and Recovery from *Pediococcus acidilactici* 3G3

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ABSTRACT

Several studies have been done in attempting to jump-start a production technology for pediocin (a "second generation", naturally occurring bacteriocin for food safety applications) derived from *Pediococcus acidilactici* 3G3, by optimizing the fermentation components and downstream process conditions using Response Surface Methodology. Recently, we have worked with rice bran-based and sucrose-based fermentation media and the use of nanozeolite for the downstream process. The experimentally-verified optimum flask fermentation conditions using a rice bran-based medium obtained from RSM were found to be: 39.4°C, 10.86% initial glucose concentration, and C-to-N ratio of 5. Cost analysis of the optimized medium had determined it to be more cost-effective than the synthetic MRS medium. In a sucrose-based medium, the lowest concentrations for Tween 80, ammonium citrate, and yeast extract were determined by Box- Behnken Design as follows: 0.5 mL/L, 0.5g/L and 5g/L, respectively, with desirability value of 0.956. Growth curve analysis using a 5-L bioreactor batch fermentation showed that the highest bacteriocin activity obtained was 204,800 AU/mL (20.37 mm ZOI) at 12th to 13th hours using *Listeria innocua* 026 as indicator organism. On the other hand, the highest cell biomass was obtained at 15th to 16th hours. Specific growth rate (μ_{max}) and doubling time (td) were computed as 0.427 h^{-1} and 1.6233 h, respectively.

The effects of contact time and nanozeolite solids loading during adsorption; and NaCl concentration and desorption time during elution, on the secondary recovery of pediocin from fermentation broth after cell

adsorption/desorption, were determined through factorial experiments. For both adsorption and elution processes, all factors considered were proven significant. Using CCD of Design Expert, the main effects (A and B), quadratic effects (A^2 and B^2) and its interactions (AB) were all proven significant in the model for both the adsorption responses and purification factor of the elution part. However, in the zone of inhibition or residual activity response, AB was insignificant. The optimum conditions generated for the numerical optimization of the adsorption runs were: 18.16 g/L nanozeolite concentration and 9.6 hours of contact time, while for the desorption runs the conditions were: 62.65 minutes of desorption time and 1.02 M NaCl. Both optimizations were considered successful since both of the responses are within the 10% prediction interval.

Economic evaluation showed that the optimum conditions provided significant savings in production and recovery costs.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Distinct Gut Microbiome of healthy Indians Living in Urban and Rural Areas

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ABSTRACT

Trillions of microbes live in the gastrointestinal tract (GIT) of human body in a finely tuned homeostatic equilibrium in the GIT ecosystem and encode key functionalities that play a crucial role in host metabolic functions, such as synthesis of macro- and micronutrients, xenobiotic metabolism, development of innate and adaptive immune systems, and resistance against invasion of enteric pathogens. The primary determinant of the composition of microbial community in human body is the anatomical location. Even in the same body site, microbial composition may vary greatly depending on the geography. The basis of this diversity is not robustly interrogated, although important role of diet, host genetics, environment, and early microbial exposure in shaping the structure of microbial ecology has been anticipated.

India, the land of extraordinary diversity, houses more than 1.2 billion people from 2000 major human communities living in approximately 1900 territorial units is the most tempting destination to study the GIT microbiome. Different factors (diets, ethnicity, geography, environmental exposure) that modulate composition of the GIT microbiota are extensively heterogeneous across the country. We observed that the GIT microbiome of Indian population living across the country are distinct compared to the other parts of the world. Multiple enteric pathogens, including *Vibrio cholerae* (*ctxAB*), Enteropathogenic *E. coli* (*EaeA*) and *Shigella dysenteriae* (*stx*), subsist in the gut of healthy Indian subjects. The abundance of such bacterial species is very low and could be identified by next generation sequencing of community microbial DNA. A detailed analysis of microbial community in the gut and the genome of pathobionts present in the gut of healthy subjects will help us to understand the importance of such bacterial species in the physiological of healthy host.

Lactic Acid Bacteria in HuangJiu and It's roles

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ABSTRACT

Lactic acid bacteria always playes an important role in the whole brewing process of HuangJiu (Chinese rice weine), but the brewers did not pay enough attention to them before. A large number of lactic acid bacteria have been growing in the rice soaking stage, usually with more than 10^6 CFU/mL, and even can reaches 10^9 CFU/mL after continuouly enrichment. There is also a large amount of lactic acid bacteria growing in the fermentation stage. Lactic acid bacteria produce lactic acid, not only to prevent the contamination of unwanted microorganisms, but more importantly, to lower the pH, to facilitate the growth of glycated microorganisms during the brewing process, and to increase the activity of amylase and protease and the like. It is also beneficial to the growth of yeast and promotes the alcohol fermentation rapidly. At the same time, it can also significantly improve the flavor of HuangJiu. The diversity analysis showed that 5 genera and 18 species of lactic acid bacteria were detected in the fermentation broth. Further studies found that *Lactobacillus plantarum, Lactobacillus mindensis, Lactobacillus brevis, Lactobacillus Hillgarii, Oenococcus oeni* can significantly reduce the content of ethyl carbonate and biogenic amines in the product, which is very important for ensuring the food safety.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Lactic Acid Bacteria and The Way Forward

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ABSTRACT

Lactic acid bacteria (LAB) play an important role in human life. They are ubiquitous and present in normal ecosystems. They involve in food preparation along the civilisation of mankind. LAB are generally regarded as safe microorganisms (GRAS), which generally known as probiotic bacterial exerting various health promoting effects, in addition to their traditional contribution in food preservation. We have explored and documented various preparations of LAB isolated from Malaysian foods. Therefore, comparative studies of various LAB preparations and their probiotic effects will be discussed in the presentation.

Keywords: Lactic acid bacteria, probiotic, postbiotics, paraprobiotic, application

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Yogyakarta, August 29-31, 2019

Characterization oF *Weissella confusa* F213 as Probiotic

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ABSTRACT

Indonesia is a tropical country where the lactic acid bacteria (LAB) is an intimate microbe involved in the fermentation of traditional fermented foods across the country. Several fermented food products especially the carbohydrate-based foods such as fermented cassava (*tape singkong*) and glutinous rice (*tape ketan*) are directly consumed without any further processing. Ecological studies on those products revealed that several LAB species such as *Pediococcus pentosaceus, Enterococcus faecium* and *Weissella confusa* were the dominant bacteria associated with those products and the life bacteria incorporated in those foods ingested by the Indonesian since long time ago. Therefore it is possible the LAB to be a part of allochthonous bacteria of Indonesian gut. *Wesisella confusa* F213 was isolated from an healthy infant feces. The strain showed good probiotic properties, survived in gatro intestinal tract of rats and could lower the blood cholesterol level when ingested in hypercholesterolemic rats and didn't cause dentrimental effects on main organ of rats. Further studies revealed that the F213 increased LAB population and improving diversity of human gut microbiota. This evident demonstrate that F213 is promising probiotic. Nevertherless, many studies are required to explore the potential used of F213 as probiotic derived from the tropics.

Keywords: Probiotics, *Weissella confusa*, traditional fermented foods

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Understanding and Perception of Mothers on Probiotic and Prebiotics in Jabodetabek area

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ABSTRACT

On line survey using Google form was conducted on understanding and perception of Mothers aged in a range of 19 – 44 years (n = 400) on Probiotic and Prebiotics and purchase intention in Jakarta and surrounding area. The interest of respondent on probiotics and prebiotics products were also surveyed. The variables were probiotic's and prebiotic's understanding, experience in buying probiotic and prebiotic, perception on probiotic and prebiotic, and purchase intention of probiotic and prebiotic. The results showed that understanding and experiences on probiotics was significantly affect ($p<0.05$) respondents' pereptions. Perceptions showed significant effect ($p<0.05$) on respondent's purchase intention. Understanding had no significant effect ($p>0.05$) on the perceptions of respondents on prebiotics, while experience significantly affect ($p<0.05$) the perception, which had a significant effect ($p<0.05$) on purchase intention of prebiotics. Respondents preferred yoghurt or milk as probiotic as well as prebiotic products, at a price of Rp. 3000-11000 with the benefit for enhancing immune response for the probiotic products, for prebiotic products was at a price of Rp. 8000-14000 with the beneficial effect for maintaining intestinal health. Mothers understood well about type and function of probiotic and prebiotic products, as shown by the correct answer obtained from 90 % of respondents. Mothers did not understood well about source of prebiotics.

Keywords: on line survey, prebiotics, understanding and perception, mothers

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

New Findings for the reason of being of Bifidobacterium in human gut

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ABSTRACT

Bifidobacterium is one of the major members of the human intestinal microbiota which comprises thousands of bacterial species. The genus *Bifidobacterium* has been known to contain > 60 species/subspecies. Some of these bifidobacteria species are typical inhabitants of the human gut (designated as human-residential bifidobacteria, HRB) whereas others naturally colonise the guts of other animals (designated as non-HRB).

This presentation will introduce the differences in genotypes and physiological characteristics between HRB and non-HRB and make some insight into for the high abundance of *Bifidobacteria* and lifespan distribution of *B. longum* in Japanese people.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Features and Signatures in Asian Microbiome: Crisis or Adaptation?

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ABSTRACT

Urbanization is occurring drastically in Asia and has a great impact on our dietary style. Asian Microbiome Project Phase III has revealed gut microbiota in Asian people altered in association with dietary urbanization. On Leyte Island in Philippines, school-age children living in urban and rural had distinct microbiota type, each classified to *Bacteroides*-enterotype and *Prevotella*-enterotype, respectively. *Bacteroides*-enterotype in urban children was associated with high-fat diet recruited in the city with Western culture. In a Thai comparative cohort study, we investigated stool metabolome in addition to stool microbiome of school-age children living in Bangkok and Buriram which is a representative rural city in Isan region in Thailand. Dietary survey indicated the distinct dietary habit between these two cities: children in Bangkok consumed much less vegetables compared to children in Buriram. The stool metabolome data indicated that Buriram children have higher level short chain fatty acids compared to Bangkok children, suggesting considerable influence of urbanized diets on metabolic activity of gut microbiota in Thai children.

We also analyzed Japanese microbiota in depth in association with daily dietary habits. As a result, 232 Japanese stool microbiome profiles were clustered into 7 groups. These microbiome groups were linked with subject dietary habits, e.g., high-fibre diet, high-fat diet, and high alcohol consumption. The high-fibre diet group kept higher level of butyrate producing bacteria, such as *Faecalibacterium prausnitzii*. High-fat diet group showed primary bile acids-dominant profile as well as lower level of butyrate in stool metabolites. This tendency was more evident in high-alcohol group.

Now we know that our gut microbiota is really sensitive to diets, notably Western diets. We should keep an eye on changing microbiota of Asians which may link with our health status.

10th Asian Conference on Lactic Acid Bacteria
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Hazards in Traditional Fermented Foods

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ABSTRACT

Food processors have a legal and moral responsibility of producing food that is safe for human consumption. Good Manufacturing Practices (GMP), a quality system with scientific evidence, is a minimum operational guideline that is required for food processors to produce wholesome, safe or unadulterated foods. It is a protection provided to the consumer from poor-quality and hazardous foods. Industrialization as manifested by an increase in food service establishment and food outlets leads to an increase in the incidence of food contaminations. In the production of traditional fermented and non-fermented foods especially in Asian countries, GMP is not much appreciated due to lack of awareness and understanding of processors on its principle and importance. In GMP, hazards are given careful attention. They refer to biological, physical and chemical hazards and allergens. In this paper, the hazards found in some selected traditional foods in Asia are discussed.

Keywords: food hazards, safe and quality foods, food fermentation, food contaminants

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Bacterial Community in Naturally Fermented Milk Products of India Revealed by Culture-dependent and Culture-independent Techniques

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ABSTRACT

Naturally fermented milk (NFM) products are popular ethnic fermented foods in India, where ethnic Indian people have invented the concept of "ethno-microbiology" involving natural fermentation of domesticated animal milk. The present study is the first to have documented the bacterial community in 54 samples of NFM products viz. *chhurpi*, *churkam, dahi* and *gheu*/*mar* by high-throughput Illumina amplicon sequencing. Metagenomic investigation showed that *Firmicutes* (*Streptococcaceae*, *Lactobacillaceae*) and *Proteobacteria* (*Acetobacteraceae*) were the two predominant members of the bacterial communities in these products. *Lactococcus lactis* and *Lactobacillus helveticus* were the predominant lactic acid bacteria while *Acetobacter* spp. and *Gluconobacter* spp. were the predominant acetic acid bacteria present in these products. Traditional knowledge of ethnic people on food fermentation has significantly contributed to enrich the dietary culture, unique culinary, nutritional security and preservation of culturable and unculturable microbiome. Application of culture-independent method mostly by high-throughput metagenomics approaches help to profile the entire microbial community and predictive functionality in some naturally fermented milk products with several health-promoting benefits to consumers.

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Lactic acid bacteria as a micro-refiner for sustainable green society

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ABSTRACT

Global optically pure lactic acid (LA) production has expanded 10-fold in the last decade mainly due to the increased demand for green biodegradable lactate polymers, the costs being competitive in the marketplace. We have developed "Designed biomass study" for fermentative production of green chemicals in the field of biorefinery (1-3). Non-edible biomass is required for design suitable for the microbes and fermentation processes. Designed biomass is a cost-competitive substrate for overall processes including fermentations, which can simplify pretreatment and hydrolysis processes for lignocellulosic biomass with less-energy and less-cost. We therefore undertook the discovery of novel promising LA producers with broader substrate metabolic capacity for utilization of designed biomass as well as the development of bioprocesses for efficient LA production. We have isolated thermotolerant *Enterococcus mundtii* QU 25, which firstly led us to the "Designed biomass study" (4-11). This presentation focuses on thermophilic *Enterococcus faecium* QU 50 recently found for improving "Designed biomass study" (12-14). QU 50 has the potential to (i) homo-ferment most lignocellulose-derived sugars to L(+)-LA at 50°C, (ii) efficiently co-ferment hexose and pentose simultaneously without carbon catabolite repression and by-products, (iii) establish non-carbon loss (high LA yield based on sugar loaded) and long-term (>20 days) non-sterilized open continuous fermentation even with mixed sugars, and (iv) greatly enhance LA yield to enzyme loading for hydrolysis of rice straw as a starting biomass by using the semi-hydrolysate rather than the glucose-oriented one in the constructed open adaptive fermentation process.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Occurrence of 10-hydroxy Octadecanoic Acid in the Cellular Fatty Acids of *Lactobacillus acetotolerans*

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ABSTRACT

Cellular fatty acid composition is known as one of the useful chemotaxonomic characteristics for bacteria because of the variety of fatty acids different from eukaryotic cells. In addition, fatty acids in bacterial cells are mostly located in cell membrane as the component of polar lipids and playing important roles in permeability of substances, holding of enzymes by maintaining suitable fluidity of the membrane. Therefore, the cellular fatty acid composition is affected by the cultivation condition.

Lactobacillus acetotolerans was isolated from turbid vinegar broth as an organism causing spoilage of vinegar fermentation in Japan. It showed high resistance to acetic acid up to 10% at pH 5.0. In the course of the study of acetic acid tolerance of *L. acetotolerans*, the effect of acetic acid concentration in cultivation media to cellular fatty acids was examined. Cellular fatty acids were liberated from freeze-dried cells cultivated in various concentration of acetic acid in cultivation media. The methyl esters of cellular fatty acids were prepared by the method of MIDI and analyzed by GC and GC/MS. When MRS was used for the basal medium, cells grown in presence of 3% sodium acetate contained more than 60% of an unidentified peak A. In contrast, cells grown in MRS without additional acetic acid did not show the peak A. When GYP was used for the basal medium, the peak A was not contained in the cellular fatty acid even in the presence of 3% sodium acetate in the medium. The possible component of the media causing this difference was Tween 80. MRS medium contained Tween 80 at 1 g/L, while GYP contained only 50 mg/L. When GYP medium supplemented with 1g/L Tween 80 was used, the peak A was found in the cells grown with 3% sodium acetate. The peak A was identified as 10 hydroxy octadecanoic acid by GC-MS in comparison with the spectrum of authentic compound. This is an interesting phenomenon of this species and may be related to the acetic acid tolerance.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

From Phylogenomics to Taxonomy: The expected taxonomic changes in the genus *Lactobacillus*

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ABSTRACT

The genus *Lactobacillus* comprises over 220 formally recognized species (http://www.bacterio.net/lactobacillus.html; as of June 2019) that are extremely diverse both from a phenotypic and a genotypic point of view, which is also reflected by the polyphyletic nature of the genus based on 16S rRNA phylogeny. They are key players in industry, food, and human and animal health-related fields. The extreme diversity of the genus *Lactobacillus* and its polyphyletic structure have been strongly suggested that this taxonomic arrangement should be re-evaluated. The need for this "taxonomic correction" has been known for a long time, but until recently, the methodologies needed to reliably group the current *Lactobacillus* species into new genera were not available.

Since 2015, *Lactobacillus* and related (e.g., *Fructobacillus*, *Leuconostoc*, *Pediococcus*, *Oenococcus*) species have been analyzed based on whole genome sequences. Both distance-based (average amino acid identity [AAI] and average nucleotide identity [ANI], as well as percentage of conserved proteins [POCP]) and sequence-based (16S rRNA gene-based phylogeny, and MLSA based on ribosomal proteins and housekeeping gene sequences) methods showed that the genus *Lactobacillus* was polyphyletic and revealed the presence of 10 robust phylogroups. The genus *Lactobacillus* could also ultimately be resolved into 10 or up to 24 phylogroups, depending on the cut-off values used for the different approaches.

In these contexts, this presentation aims to introduce the current progress status on research analysis in terms of reclassification of the genus *Lactobacillus*.

Keywords: *Lactobacillus*, phylogenomics, whole genome sequence, reclassification, new genera

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Probiotic Application to Prevent Autoimmune and Neurodegenerative Diseases

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ABSTRACT

The gut microbiota is vital to wellness and has lately become an aim for live bacterial cell biotherapies for numerous chronic diseases including neurodegenerative disease. There are three basic mechanisms that mediate the communication between the gut and the brain: direct neuronal communication, endocrine signaling mediators and the immune system. We investigated the effects of different probiotic bacteria on Murine and individual model of Parkinson (PD) and Multiple sclerosis (MS). *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum* and *Lactobacillus fermentum*, each with the concentration of 2×10^9 CFU/g were determined in clinical trial studies and *Bacillus coagulans, Lactobacillus casei, Lactobacillus paracasei, pediococcus acidilactici* and *lactobacillus brevis* each with a concentration of 1×10^9 CFU/ml were administered in murine studies. We found that probiotic intake after 12 weeks in MS subjects had favorable effects on EDSS, parameters of mental health, inflammatory factors, markers of insulin resistance, HDL-cholesterol, total-/HDL-cholesterol and MDA concentrations, but unchanged other biomarkers of oxidative stress, FPG and other lipid profiles. In murine research, the result of the oral bacteria's administration shows that there are some significant differences in inflammatory and anti-inflammatory factors between experimental group and MS control group. Also, we determined the effect of consuming probiotic on movement and metabolic parameters in people with PD. We realized that taking probiotic for 12 weeks by people with PD had favorable impacts on MDS-UPDRS, hs-CRP, GSH, MDA and insulin metabolism, but did not affect other metabolic parameters. The other results from murine study of Parkinson disease demonstrate that 4 weeks intake of probiotic has some effects on behavioral factors. These studies demonstrated that consumption of daily probiotic can prevent or improvement the PD or MS diseases.

Keywords: Probiotic, MS, Parkinson, Inflammation

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Yogyakarta, August 29-31, 2019

Alterations of Gut Microbiota Associated with Distinct Allergic Phenotypes: A longitudinal Cohort Study of Thai infants

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ABSTRACT

There is dramatic increase in incidence of allergic diseases in Asia. Allergic diseases usually originate in early life. Throughout the first 3 years of life, the maturation of immune system occurred along with the development of gut microbiome. Notably, most studies are conducted in cross-section and there are few reports in Asia. This study aimed to determine the association of microbial colonization with allergic diseases compared to healthy individuals in an Asian birth cohort. Population-based birth cohort study from July 2016 to May 2018 in Bangkok, Thailand. Total 336 children had follow-up visits until 24 months of age. The incidence of allergic diseases was 12.5% contained 3 atopic phenotypes including atopic dermatitis (AD) 58.8%, food allergy)FA(17.7% and both atopic dermatitis

and food allergy)AD/FA(23.5%. At 9-12 months, Shannon index revealed that diversity index of FA samples was the lowest at 2.08 whereas the others were in range of 2.32-2.38. *Lachnospiraceae* was the most abundance in all groups. Notably, the abundance of three families, namely *Bifidobacteriaceae, Enterobacteriaceae* and *Erysipelotrichaceae* significantly differed among the groups $)p < 0.05($. The amount of *Erysipelotrichaceae* in AD and AD/FA groups was significantly higher than controls $)p<0.05($. *Erysipelotrichaceae* was suggested to correlate with inflammation-associated diseases in human. The relative abundance of *Enterobacteriaceae* in FA was slightly higher than control. While *Bifidobacteriaceae* in control was the highest. This finding serves for further study in longitudinal birth cohort at 18 and 24 months. Understanding the dynamic of microbial colonization patterns in allergic diseases will lead to discovering a promising clinical target for allergy prevention and treatment.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Lactobacillus plantarum DR7 and the Brain: Evidence and Mechanisms

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ABSTRACT

Probiotic microorganisms have a long history of safe use and have been documented for their various health benefits. Many strains of lactic acid bacteria (LAB) from the genera of *Lactobacillus* have been identified to possess probiotic properties. They have been much emphasized on their roles to regulate gut well-being, including the alleviation of lactose intolerance, improvement of diarrhoea and inhibition towards pathogenic bacteria in the gut. Recent evidences have illustrated the potential of LAB for brain health, ranging from neurodegenerative diseases to stress, memory and cognition. *Lactobacillus plantarum* DR7, a cow's milk isolate from Penang, Malaysia has shown a reversal of symptoms associated with Alzheimer's Disease in a fruit fly model, accompanied by distinct changes of gut microbiota profiles. In aging and hyperlipidemia rats, DR7 has shown improved stress and anxiety symptoms, accompanied by improved memory. The administration of DR7 for 12-weeks in stressed adults has contributed to improve stress and anxiety scores, in addition to memory and cognitive abilities, primarily via enhancement along the serotonin pathway and maintenance of the dopamine pathway. My talk will highlight the evidence of a probiotic strain, primarily targeting brain health, via models ranging from insects to human.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Subspecies Identification and Medium Optimization of Two Strains of Probiotic *Lactobacillus kefiranofaciens*

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ABSTRACT

Lactobacillus kefiranofaciens has two subspecies, *kefiranofaciens* and *kefirgranum*, with the distinct phenotypic features. However, high similarity of genetic background between these two subspecies makes accurate and definitive identification become a very challenging task. Our lab previously isolated two *L. kefiranofaciens* strains from kefir grains. For classification of subspecies, numerous approaches, including morphological characterization, ERIC-PCR and RAPD fingerprinting, 16S rRNA and housekeeping gene sequence analysis, carbohydrate fermentation tests, and whole cell protein profiling, were conducted by comparison with two type strains (*L. kefiranofaciens* subsp. *kefiranofaciens* BCRC 16059^T and *L. kefiranofaciens* subsp. *kefirgranum* BCRC 80410^T). Results indicated that both strains were related to subspecies *kefirgranum* based on the findings of the RAPD fingerprinting and whole cell protein profiling. After taxonomy of subspecies, optimal culture conditions for *L. kefiranofaciens* were determined. We observed a significantly higher growth at the temperature of 30°C, initial pH of 6.0 without reducing agent. The best carbon and nitrogen sources for growth medium was glucose and yeast extract, respectively, with supplements of skim milk and calcium. Subsequently, response surface methodology (RSM) was carried out to further maximize the yield of biomass. Results showed that the biomass of this optimized medium increased 15.1-fold than regular MRS medium after RSM optimization.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

The Growing Burden of Antibiotic Resistance – Can Probiotics Help in Reducing the Crisis?

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ABSTRACT

Antibiotics are the greatest discovery of the 20th century and have played a significant role in reducing the global burden of infectious diseases. However, their extensive and inappropriate use has resulted in the emergence of Antibiotic Resistance which has threatened the ability to treat disease. The 21st century has witnessed critical concerns about the development of Methicillin Resistant *Staphylococcus aureus*, MDR and XDR Mycobacteria, ESBL producing Gram Negative bacteria, NDM-1 producing Enterobacteriaceae, multi drug resistant Salmonella and Enterococci. The rapid spread of Antibiotic resistance has negatively impacted surgical procedures, organ transplantation, treatment of preterm babies and cancer. Further several lines of evidence confirm that antibiotic administration can result in dysbiosis of the intestinal microbiota. The alteration in the intestinal microbiota has been associated with atopic, inflammatory and autoimmune disorders and an increased susceptibility to disease. Thus, combating antibiotic resistance is an urgent global public health concern.

In light of this knowledge, alternatives to antibiotics are urgently needed to combat Antibiotic resistance. Probiotics is one such alternative that can favourably modulate and stabilize the intestinal microbiota and provide colonization resistance against pathogenic organisms. The rapidly growing interest in probiotics and discourse has paralleled the dramatic increase in attention given to antibiotic residence from the early 1990s onward. Probiotics are an excellent means of remediation and deterrence against a variety of intestinal disorders and extra-intestinal infections. Decades of research have identified some promising gastrointestinal and immune targets for probiotics that include maintenance of a healthy intestinal function, improved tolerance to antibiotics and an overall reduced risk for different chronic diseases. The renewed interest in probiotics brings new hope for combating disease causing bacteria while limiting collateral damage to the microbiota.

The Skin Gut Axis in Skin health and disease: A probiotic paradigm with therapeutic implications of Psoriasis and Atopic Dermatitis

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ABSTRACT

We focus on the gut microbiota and the role of microbiome as potential principle actors along the gut-skin axis. We correlate loss of specific bacterial taxa with development of skin disease of Psoriasis and Atopic Dermatitis, by treating mice with probiotics that result in distinct microbiota changes and lead to symptom improvement of skin disease**.** *Lactobacillus sakei* Probio-65 isolated from Kimchi, exhibited skin probiotic properties and showed good antibacterial activity against a variety of pathogenic bacteria. *L. sakei* Probio-65 has an inhibitory effect on atopic dermatitis and psoriasis-like skin lesions, and may represent an effective new anti-inflammatory agent through both oral administration and skin topical application.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Potentiality *Lactobacillus plantarum* group for probiotic functionality

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ABSTRACT

The potential health benefits of probiotics has gained increasing attention in a health conscious society from the days, when Eli Metchnikoff hypothesized that the lactic fermented dairy products are associated with gut microbial homeostasis. By definition, probiotics are live microorganisms that confer health benefits on the host in a safe and efficacious manner when administered in adequate amounts. The beneficial effects of probiotic bacteria on human health are now widely accepted, and include the production of range of antimicrobial substances, suppression of the growth of pathogenic and putrefying bacteria, and modulation of the host immune system. Therefore, probiotics have become widely available commercially. Indian probiotic market is emerging as one of the most high growth (20%) potential markets worldwide due to multiple factors such as growing health concerns among consumers etc. AS per the recent report, availability of probiotic is also increasing gradually due to expanding distribution channels across the country, which is steadily bridging the demand-supply gap.

Probiotics market was estimated of $45.64 billion in 2017 and projected to grow at a CAGR of 7.0% to reach $64.02 billion by 2022. This has been driven by the growing awareness about the benefits of probiotics among consumers. Most probiotics are being consumed in the form of capsules or tables. However supplementation through fermented foods has received attention that constitute an important delivery for pre and probiotics help for their in situ enrichment, biofortification of vitamins and minerals. Presently, probiotics comprises only a species of lactobacilli and bifidobacteria, most part of gut microbiota and nutritional requirement yet to be characterized.

Several lactic acid bacteria (LAB) have purported to exert many health benefits (like anti-oxidant, anti-cholesterol, bio-protective,

immunomodulating etc.) and have been administered in food preservation and fermentation processes. Among the LAB, *Lactobacillus plantarum* has gained more attention due to its probiotic application in many food products. Numerous strains of *L. plantarum* that are used in fermentation and preservation of dairy, plant materials like *Kimchi*, pickles, silage, cassava etc., are well characterized and establish to show variable phenotypic and diverse properties. However, the current taxonomy of *L. plantarum*- group (LPG) has phylogenetically closely related subspecies, which include *Lactobacillus paraplantarum*, *Lactobacillus pentosus*, *Lactobacillus arizonensis, L. plantarum* subsp. *plantarum, L. plantarum* subsp. *argentoratensis, L. xiangfangensis* and *L. fabifermentans.*

In our laboratory, we have characterized *L. plantarum* obtained from fermented vegetables for their adhesion genes (*fbp* and *mub*). The detection of novel alleles among the isolates of fermented vegetables suggested that this food niche can act as an ideal source for probiotics. The LPG isolates exhibiting deletion, duplication, and speciation events in the mucin binding adhesion (MUB) domain repeats, suggest inter-strain diversity. A study on MucBP domain repeats among LPG strains showed genetic diversity due to the detection of integrase and transposases flanking to the *mub* gene among *L. plantarum* and *L. paraplantarum* strains. This could be the first study where the naturally prevailing higher number of MucBP domains contributed for better expression and thereby explains its strain-specific domain composition. The presence of transposases belonging to *IS*256 and *IS*5 family helps in understanding the genetic recombination events in an ecological niche that might lead to intra-species/intra-strain microbes. Our further investigation on MucBP domain repeats from D_7-D_{10} suggested their evolution among *L. spicheri, L. namurensis* and *L. hammesii* and proposes that these fermented foods provide a natural environment for genetic recombination. Many strains of *Lactobacillus plantarum* have conferred their health benefits by adhering to the intestinal epithelial layer, release of certain cytokines, excluding pathogens etc. We reported, the pro- and anti-inflammatory cytokines gene expression studies were found to play a major role in immunomodulation of Caco-2 cells on treatment with *Lactobacillus* species. Among all the cultures studied, *Lactobacillus paraplantarum* MTCC 9483 and *L. plantarum* subsp. *plantarum* CFR MFT1 was found to be a potent immune stimulant during oxidative stress, LPS-induced and pathogen invasion conditions with more adherence ability. These cultures have shown more adhesion and down-regulated some of the inflammatory cytokines

such as tumour necrosis factor, range of interleukins as well as transforming growth factors. Our findings suggestsenvironmental niche would have facilitated the divergence among isolates studied. The cultures from *sinki* were found to be quite divergent in all the potential probiotic properties evaluated. Further, the different strains of LPG from fermented foods harbored the *pln* locus with genetic diversity. These indigenous LPG strains were found to have good functional attributes like anti-oxidant, anti-cholesterol, and anti-microbial activity. Because of the presence of these properties, the LPG strains can be a good probiotic starter culture protecting the intestinal mucosal layer from the invading pathogenic microbes as observed for their better adhesion to Caco-2 cell line.

This lecture focuses on our efforts in identifying probiotics strains of *Lactobacillus plantarum* group from vegetable origin, and their health claim, their functionalities, *in vitro* and *in vivo* characteristics, cell line studies etc. It also explains our new approach to explore and supplement probiotics through fermented food on non-dairy origin.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Intestinal adsorption of glucose, cholesterol and bile salt by simultaneous incorporation of edible microbiosorbent and intestinal bacteria

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ABSTRACT

Natural edible adsorbents can play an important role in human healthcare via lowering the harmful materials from gut and regulating the microflora composition. In this work, several microalgae and plant sources, including *Chlorella vulgaris* (Ch), zucchini (Zu), ginger (Gi), rice bran (RB) and Zahedi date (ZD), were studied on the production of edible and bacterial biosorbents for the adsorption of glucose, cholesterol and bile salt *in vitro*. By screening the sources, Ch, Zu and Gi were selected for maximizing the production of *Lactobacillus plantarum.* Afterward, the optimized mixture of zucchini-ginger-*C. vulgaris* (ZuGiCh), obtained from the combination of the microparticles of zucchini (48.80 g/l), ginger (35.00 g/l) and *C. vulgaris* (39.30 g/l), maximized *L. plantarum* cells to 2.37 ± 1.86 $\times 10^9$ CFU/ml and minimized *Escherichia coli* and *Staphylococcus aureus* cells to 4.53 ± 0.41 $\times 10^8$ and 8.10 ± 0.61 $\times 10^8$ CFU/ml, respectively. The simultaneous incorporation of *S. aureus* and ZuGiCh as an edible microbiosorbent resulted in the highest adsorption of glucose (57.65%) and cholesterol (83.65%) at initial concentration of 40 g/l adsorbate while the incorporation of *L. plantarum* and ZuGiCh showed the highest adsorption of bile salt (42.59%) at initial concentration of 80 g/l bile salt. The ZuGiCh microparticles can be used as a potentially intestinal adsorbent for improving microflora and lowering of health hazards from gut.

Keywords: Microbiosorbent, Adsorption, Cholesterol, Probiotic, Microalgae, *Lactobacillus plantarum.*

Restoration of Gut Microbiota by Administering Female Rat with LAB Culture Supernatants

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ABSTRACT

Oriental medicinal herbs have long been consumed but often cause side effects manifesting diarrhea and allergic reactions. Phytochemicals in the medicinal herbs are commonly found as antioxidants and antibacterial compounds. It is indicated that the antioxidants could reduce the diversity of gut microflora. However, the direct association between ingredients of medicinal herbs and gut microflora remains uncertain in human intestine. To explore the mechanism underlined, samples of the hot water extracts of medicinal herbs were tested in vitro for their activity against common microorganisms (*Lactobacillus plantarum, E. coli* :O6*, Staphylococcus aureus, Bacillus cereus*) by monitoring growth of the bacterial strains tested. To investigate the restoration of gut microbiota affected by treating medicinal herbs, feces samples have been examined for 4 weeks after female rats were administered the water extract. PCR-DGGE data have shown that diversity of gut bacterial flora was apparently reduced. However, when those were treated with culture supernatants of LAB, beneficial bacteria, such as *Lactobacillus* sp. were selectively stimulated while potential harmful bacteria were decreased. Taken together, our experimental data suggest that the gut microbiota was significantly modified by administrating the water extracts of medicinal herbs alone or together with culture supernatants of LAB. Therefore, the culture supernatants may be applied to balance the impaired gut microbiota after consuming oriental herb extracts.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

"Recent Strategy of Development of New Functional Yogurts Using Probiotics in Japan"

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ABSTRACT

The field of probiotics has advanced considerably in recent years, spurred by global progress in understanding the role of the human microbiota in health and disease and the need to define effective strategies to shape a healthier microbiota. Recently, many systematic reviews and meta-analyses provide convincing evidence of the benefits of probiotics.

As probiotics, lactic acid bacteria (LAB) and Bifidobacteria (BF) have been investigated as starter strains for functional yogurts. In Japan (1991), "the Food for Specified Health Uses" (FORSHU, Japanese name TOKUHO) system was inaugurated as the world's first approval system on health claim labeling for food products. There are 17 health claim categories and the foods to modify gastrointestinal conditions (300 products) including probiotic yogurt are most popular in Japan. In total, 1,065 products are approved under FOSHU as of June, 2019. The market scale of fermented milk products including functional yogurt from 2006 to 2019 was compared by the data of a sum of money and category of yogurt. In 2015, a new system of "Foods with Function Claims" is proposed by Consumer Affairs Agency. In Japan, the third probiotic boom may have been started. As the market scale of yogurt increase in these 5 years, the break-through of 430 billion yen (35 billion USD) must be true.

Japanese functional yogurts are classified into 3 big categories such as Functional LAB type yogurt, Functional materials type yogurt and others. As the present topic yogurt in Japan, Probio Yogurt LG21 (exclusion of *Helicobacter pylori* from stomach and reduce inflammation level), R-1 yogurt (phosphate exopolysaccharide: EPS activate NK cells through IFN-gamma

secretion) and PA-3 yogurt (prevent gout by reducing uric acid in blood serum) by Meiji HD and anti-allergy yogurt by Takanashi and Calpis.

Many functions are considered in the selection of probiotics including competitive adhesion and exclusion of enteritic pathogens, cholesterol lowering effects, and positive and negative immuno-modulatory effects. Adhesive activity to the human intestine is one of the most important characteristics of probiotic LAB/BF. Recently, we developed a new screening assay using the BIACORE and found "blood type LAB" expected to improve gastrointestinal health by continuous proliferation in colon and removal of harmful bacteria causing inflammatory bowel disease (IBD) such as UC/CD. Moreover, new yogurts in the future were introduced such as anti-IBD yogurts. Most recently, we have started the new study about the removal of sulfate reducing bacteria (SRB) from human intestine by using BF to prevent UC.

In future, the increase of development of symbiotic yogurt by fermentation or addition of the combination of LAB/BF and by addition of several prebiotics (GOS: galacto OS, Lactulose, Fructo OS, lacto-sucrose) and newly developed oligosaccharides (fucosyl and sialyl oligosaccharide) as FOSHU products is presumed.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

The Count Counts: Applications of Yakult Intestinal Flora-Scan (YIF-SCAN) to Intestinal Microbiome Research

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ABSTRACT

Alterations in the intestinal microbiome have been linked to various health status, ranging from intestinal diseases to mental disorders. Probiotics represented by lactic acid bacteria have been studied to seek its impact on the intestinal microbiome and the associated host health and disease. Current gold standard of microbiome analysis is the next generation sequencing (NGS) which provides proportional information of the microbial communities. However, recent studies suggested that absolute bacterial load is indispensable to decipher genuine features of microbial ecosystem as well as its interaction with host, as exemplified by a "low-cell-count *Bacteroides* enterotype" reported by Vandeputte et al. Statistical complexity is another problem as proportional data cannot be directly applied to standard statistical analysis. Yakult Intestinal Flora-Scan (YIF-SCAN) is a highly sensitive quantitative analytical system that is based on reverse transcription-quantitative PCR (RT-qPCR) targeting microbial rRNA molecules. The system enables absolute quantification of more than 70 target bacterial groups belonging to various human gut bacterial clades, including predominant obligate and facultative anerobes. YIF-SCAN is validated through the whole process of stool collection, storage, isolation of fecal RNA, preparation of reaction microplate for PCR, RT-qPCR, and calculation of bacterial count from PCR data, which strongly enhance comparability and reproducibility among different research. In this context, application of this system in population studies, clinical diagnosis and intervention trials will be introduced together with found solid evidence of *Lactobacillus casei* strain Shirota. The results suggest the importance of future microbiome research to much care about bacterial absolute count in the ecosystem.

Keywords: Intestinal microbiome, YIF-SCAN, Lactobacillus, Next generation sequencing

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Yogyakarta, August 29-31, 2019

Are Fermented Foods Still a Promising Source of Beneficial LAB?

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ABSTRACT

Although phylogenetically coherent, the lactic acid bacteria (LAB) are a physiologically diverse group within six families and at least 40 genera under the Phylum Firmicutes. Generally fastidious in nature, they are distributed among a wide variety of ecosystems including fermented foods and the gastro-intestinal tract (GIT) of man and animals. Thanks to their beneficial role in food fermentations, the LAB traditionally enjoy a positive image throughout human history and domestication. Even when the association of different LAB taxa with fermented foods has been reported, strains of only a few LAB species usually dominate fermenting food ecosystems, and, as a result, have found application in food fermentations and related processes (e.g., bio-preservation). On the other hand, an increasing variety of LAB species and strains are presently finding the way into the rapidly growing probiotic market; especially for this area, traditional fermented foods comprise an exciting and most promising "prospecting" field for new strains.

Showing great potential as cell factories for the chemical industry, molecular biology is opening ways for targeted selection of LAB strains for conventional and new applications. Based on their genetic make-up and physiological capabilities, the LAB have developed over time to become specialists, partly with strict adaptation to a variety of distinctive niches. This provides a valuable basis for developing biotechnical processes from renewable resources. By their fermentative nature the LAB are characterised by high carbon uptake rates, strictly regulated and simple metabolic pathways with few metabolites, and by low biomass formation. Using metabolic engineering for strain improvement new ways have been opened for the production of ethanol, butanol, diacetyl, and also a range of

nutraceuticals, sweeteners and vitamins. However, industrial applications will eventually be determined by economic factors.

The area of probiotics represents a major field of recent and projected future developments. Several factors will determine the future success of LAB probiotics. In particular it has become imperative to clarify the basic mechanisms underlying their postulated beneficial functions, e.g., by using both validated *in vitro* and *in vivo* models and involving meta-omics approaches. Final confirmation should be on the basis of double-blind randomized clinical trials. Further important issues include (a) the fine-tuning and defining of intra-species diversity, (b) developing of validated *in vitro* models for selection of personalised probiotics, and (c) novel beneficial applications within the extra-intestinal microbiome.

Keywords: fermented foods; lactic acid bacteria; beneficial functions; probiotics

Role of lactic Acid Bacteria as Probiotics in the Rumen Fermentation

Yantyati Widyastuti

Probiotics which can also be described as 'direct fed microorganism' is live microorganisms in the rumen that confer a health benefit on the host. Lactic acid bacteria are potential as probiotics for ruminant. Selection of bacterial probiotics is the most important step in developing probiotics product, at least the bacteria show ability to survive and give positive effect in the rumen fermentation. Gas production from in vitro rumen fermentation can be used to select potential strains of bacteria for probiotics during selection. Evaluation or effect of probiotics in the rumen was mainly detected by production of volatile fatty acids.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Psychobiotics as Biotherapeutic Agents for Neurodegenerative Disorders

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ABSTRACT

Recent studies have revealed that gut microbiota influence neurodevelopment, modulate behavior, and contribute to neurological disorders through microbiome-gut-brain axis (MGBA). Psychobiotics, a class of probiotics with psychotropic activities, integrate neural, hormonal, and immunological signaling via the gut-brain axis to combat a broad spectrum of complex diseases including mental illness, irritable bowel syndrome, neurodegenerative diseases and neurodevelopmental disorders. We evaluate the effects of PS128 in animal models of Alzheimer's (AD) and Parkinson's disease (PD). IntracerebroventricularI streptozotocin (icv-STZ) injection was conducted in triple transgenic AD mice, as a model of early-onset family AD, and C57BL/6J, as a model of late-onset sporadic AD. From behavioral results, we found that icv-STZ caused mouse deficit in spatial learning and long-term memory extraction in B6 mice, and cognitive function in 3xTg-AD mice. The pretreatment of PS128 ameliorated the cognitive deficit caused by icv-STZ in both AD models. In the MPTP-induced PD-like mouse model, pretreatment of PS128 alleviated the motor deficits, protected the dopaminergic neurons both in substantia nigra and striatum, ameliorated the monoamines reduction, α-synuclein accumulation and glial cell activation in striatum, and suppressed MPTP-induced elevation of TNF-α, IL-1β, IL-6 in striatum and midbrain regions. In the 6-OHDA rat hemi-PD model, pretreatment of PS128 suppressed the M1 beta-oscillation and improved the locomoter activity in stepping test. Our study demonstrates psychobiotic PS128 could be a potent alternative for the treatment of neurodegenerative diseases, including PD and AD.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Asian Microbiome Project Phase III: Infant microbiome

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ABSTRACT

The pioneer microbes colonizing the gastrointestinal tract (GI) arrived through vertical transmission from the mother prior to and during birth. We characterized microbiota in the feces, vagina and breast milk of Indonesian mothers and microbiota of feces and saliva of their infants at birth and up to 48-months. Next generation sequencing was used and the results correlated with food surveys after weaning. The predominant fecal microbiota of mothers was *Prevotella* with low abundance of *Bifidobacteria*, whereas that of the infants at birth (before one week) until weaning (12 months) was *Bacteroides-Bifidobacteria*. The microbiota profile of infants and mothers converged after weaning to become *Prevotella*-type. *Bacteroides-Bifidobacteria* predominance was established rapidly (within one week) in the GI of infants after birth. The study provides a model for the understanding of transition and establishment of human GI microbiome. *Bacteroides* and *Bifidobacteria* may be the favored GI-bacteria among pre-weaned infants of all mothers, both the *Prevotella*- and *Bacteroides-Bifidobacteria* type. This study shows that it is the intrinsic GI environment rather than abundance of entering microbes that determines the infant microbiota profile. Before weaning, the abundance of the commensal bacteria was inversely correlated with potential pathogens in GI. The commensal may have protected the infants against pathogens. The abundance of commensal bacteria in the infants after weaning was largely positively correlated with opportunistic pathogens up to 24 months age. Cytokines related to responses to microbial infection in fecal water were high after weaning. Immunity again infection built up at around the time of weaning may protect the infants again infection from then on. The abundance of *Prevotella* in infants after weaning was correlated with

frequency of consumption of carbohydrates, legumes and fruits; while the abundance of *Bifidobacterium* was inversely corrected with Indonesian vegetable, fruits and seasonings. At 48 months, a balanced adult-like GI microbiome and immunity established while the correlation between commensal and potential pathogens was weak.

ABSTRACTS OF ORAL PRESENTERS

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Lactococcus lactis Producing Phage Lysins as Potential Enzybiotics Against Methicilin Resistant *Staphylococcus aureus*

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ABSTRACT

Phage therapy is a potential alternative solution to the global antimicrobial resistance problem currently plaguing mankind. Bacteriophages are specific viruses which only attacks bacteria. They are able to lyse the bacteria cells through the production of lysin enzymes which are cell wall hydrolases that breaks down specific bonds in the peptidoglycan of the target bacteria. These lysins, also known as enzybiotics, can be broadly categorized into virion associated peptidoglycan hydrolases (VAPGH) or endolysins. The former locally degrade the bacteria cells during entry of the phage while the latter lyse the cells during release of new progenies at the end of the replication cycle. In this study, lysins from Phage 88 against Methicillin-resistant *Staphylococcus aureus* (MRSA) was cloned and expressed in *Lactococcus lactis*. Methicillin-resistant *Staphylococcus aureus* (MRSA) are resistant to many types of antibiotics, causing complications in treatment. While usually associated with the skin, it also causes other diseases ranging from septic shock to food poisoning. *L. lactis*, a generally regarded as safe (GRAS) bacteria have been extensively used as a model for genetic engineering in lactic acid bacteria and is extensively researched as a

gastrointestinal delivery vehicle. The recombinant lysins showed lysis activity on plate assays, observed by halozone formation. These lysins were further characterized for its antimicrobial activity. Additionally, the recombinant *L. lactis* expressing phage lysin is expected to function as an antimicrobial agent against MRSA when grown in co-culture.

Keywords: phage therapy, lysins, *Lactococcus lactis,* MRSA, antibiotic resistance

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Bile Salt Hydrolase Activities of Probiotic Lactic Acid Bacteria: Desireable or Undesireable?

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ABSTRACT

Cholesterol lowering effects have more been linked to bacterial bile salt hydrolase (BSH) activity, which is found among various Gram-positive intestinal bacteria, such as Enterococcus, Bifidobacterium, Clostridium and Lactobacillus. The two predominant bacterial modifications of bile salts include: (1) hydrolysis of glycocholic acid into cholic acid, which may contribute to lower cholesterol levels and (2) dehydroxylation of cholic acid into deoxycholic acid acid, which surprisingly could function as procarcinogenic substance. This paper will discuss about the two faces of BSH activities and recent knowledge for prevention of possible side effects associated with BSH activity.

Keywords: lactic acid bacteria, bile salt hydrolase activity, probiotic

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Identification of Probiotic Potential *Lactobacillus* from *Mandai* Using Molecular Technique

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ABSTRACT

To date, lactic acid bacteria (LAB) still become the most beneficial microorganisms due to their probiotic potential and inhibitory activities such as antimicrobial production which able to inhibit the growth of spoilage microorganisms and pathogenic bacteria, thus can maintain the hygienic and quality of the products or host health. In Indonesia, fermented traditional foods such as *tempoyak*, *bekasam*, and *sayur asin* have been found to be the habitats of probiotic LAB. In this study, *mandai,* the traditional fermented food which made from fermentation of *cempedak* (*Artocarpus champeden* Spreng) *dami* was explored. The aim of this study is to identify probiotic potential *Lactobacillus* from *mandai* (traditional fermented product from *dami* of *Cempedak*) using molecular technique (DNA fingerprinting). The methods used in this study include: screening probiotic *Lactobacillus* and molecular identification of potential *Lactobacillus*. Based on probiotic screening, 26 *Lactobacillus* isolates were categorized as acid tolerance and 17 isolates were considered as bile salt tolerance bacteria. Fifteen *Lactobacillus* isolates demonstrated excellent inhibitory properties against indicator bacteria. Due to partial sequences, all identified genus of *Lactobacillus* were still unconfirmed, however, based on phylogenetic tree isolate A14 had the most similarity with *Lactobacillus vaccinostercus* NRIC 0624. Isolate B30 had similarities with *Lactobacillus harbinensis* and *Lactobacillus perolens*. Isolate F71 had similarities with *Lactobacillus casei* and *Lactobacillus paracasei.*

Keywords: Lactic Acid Bacteria, *Lactobacillus,* probiotic, identification

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Effect of D-Tagatose as Substrate on The *Lactobacillus bulgaricus* Existed Medium

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ABSTRACT

D-tagatose is low calorie rare sugar that has been known as similar sweetening properties as sucrose and might produce the various product benefit in food. This research was done to analyze MRS medium containing *Lactobacillus bulgaricus*. The change in pH and the physical appearance of final MRS was studied. The incubation time was conducted for 6 hour and the temperature of 35˚C was used. As result, this lactic acid bacteria might change slightly the pH during incubation. It could be differentiated between the treatments without and with D-tagatose. The remarkable appearance was also able to be distinguished among the treatment with and without D-tagatose. As conclusion, D-tagatose might induce the pH change and physical appearance of the MRS medium containing *Lactobacillus bulgaricus*. This finding might provide information to the use of this rare sugar for determining the pH and physical appearance at final product.

Keywords: *L. bulgaricus*, D-tagatose, pH, physical appearance, incubation.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Rehydration Improves Probiotic Properties of Lyophilized *Lactobacillus plantarum*

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ABSTRACT

Lyophilization is a process that allows stable preservation of starter cultures and probiotics for extended periods of time. During lyophilization, bacteria are exposed to different stress conditions such as high osmotic pressure (due to the high concentration of the solutions used as cryoprotectants), low temperatures (freezing), and low water activity (drying). Those factors make the cells more vulnerable to the conditions of the upper gastro-intestinal tract to which they are subjected after consumption. On the other hand, the common agreement for a probiotic definition states that the microorganism should be alive in order to exert a beneficial effect on host health. In the present work, the rehydration in the wet state of freeze-dried probiotic preparations before consumption is proposed as a possible alternative to enhance the metabolic functionality such as viability in the G1 tract and cell adhesion *in vitro* to intestinal epithelial, Caco2, cells. Properties that can be compromised due to the freeze-drying process. The results indicated an increase in the cell viability after GI tract pass as well as in the cell adhesion of the rehydrated cells similar to the fresh overnight cultures. Our findings suggest that the use of an adequate rehydration solution for activating putative probiotic strains such as *Lactobacillus plantarum* can improve a strain's functionality compared to the non-rehydrated (freeze-dried) microorganism.

Keywords: Freeze-drying, rehydration, survival, functionality

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Investigation of Antioxidant Activity and Cholesterol Reducing Ability from Lactic Acid Bacteria AND *Bacillus* spp. Isolated from Foods and Fish Samples

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ABSTRACT

The basic criteria for selection of probiotics described by WHO include several properties such as antimicrobial activity, tolerance to gastrointestinal tract conditions, epithelial adhesion ability and functional properties. This study aimed to investigate antioxidant activity and cholesterol reducing ability of lactic acid bacteria and *Bacillus* spp. isolated from fermented foods and fish intestines. In total, 110 lactic acid bacteria and 47 *Bacillus* spp. were examined for two functional properties. Antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl)DPPH(assay. Cholesterol reducing ability was evaluated by determining the remained cholesterol in the spent medium using o-phthaladehyde method. It was found that *Bacillus aerius* B81e, *B. polymyxa, Bacillus* sp. B85b, *Bacillus* sp. strain 24 together with *Lactobacillus plantarum* CR1T5 and *Lactobacillus* spp. strains L42d, L42c, L42u, L42i, L42j, L42p, L42q displayed high DPPH scavenging activities)>30 %(. Meanwhile, *B. polymyxa, B. aerius* B81e, and *Bacillus* spp. strains 24, B79a, B51f, B83c, B39d, B78e could reduce more than 60% cholesterol whereas *Lactobacillus* strains L42d, L42u, L42q, LAB40 reduce more than 40% cholesterol. Interestingly, *B. polymyxa*, *B. aerius* B81e, *Bacillus* sp. strain 24 together with *Lactobacillus* strains L42d, L42u and L42q possessed both antioxidant activity and cholesterol reducing ability. Some of these bacterial strains have already been proved as probiotic bacteria. Therefore, these findings reveal the promising bacterial probiotics to be employed in functional foods.

Keywords: antioxidant, cholesterol reducing, lactic acid bacteria, *Bacillus*, probiotic

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Yogyakarta, August 29-31, 2019

Investigation on The Responses of *Lactobacillus mali* APS1 to Environmental Stresses and Its Survival After Freeze-Drying

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ABSTRACT

Lactobacillus mali APS1, a potential probiotic strain, has been demonstrated to possess immune-regulatory, anti-colitis and anti-obesity effects *in vitro* and *in vivo* in our lab. To improve survivability of *L. mali* APS1 under severe stressful conditions, we investigated the adaptive responses of *L. mali* APS1 to various environmental stresses. The previous results showed that the sublethal and lethal levels of cold, heat, acid and bile salts for *L. mali* APS1 were 10°C and -20°C, 42°C and 52°C, pH 3.5 and 2.0, and 0.1% and 2%, respectively. Adaptation of *L. mali* APS1 to cold, heat and bile salts induced homologous tolerance. In the present study, we further evaluated the protein expression of *L. mali* APS1 influenced by stress adaptation by two dimensional gel electrophoresis (2-DE). The expression levels of 14, 13, 8, and 16 proteins in cells of *L. mali* APS1 were altered by cold, heat, acid and bile salt adaptations, respectively. After freeze drying, the survival rates between stress-adapted and non-adapted cells of *L. mali* APS1 were not significantly different. Stress-adapted *L. mali* APS1 maintained higher bacterial counts during subsequent storage at -20 and 4°C. Our study revealed that stress adaptation could be a possible strategy to improve the survival of *L. mali* APS1 under environmental stress conditions.

Keywords: *Lactobacillus mali* APS1, stress adaptation, homologous tolerance, two dimensional gel electrophoresis (2-DE), freeze drying

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Isolation of *Bifidobacterium* from Infant's Feces on Its Antimicrobial Activity

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ABSTRACT

Bifidobacterium are a group of Lactic Acid Bacteria (LAB) that commonly found in the gastrointestinal tract and vagina. LAB has many health benefit, such as produce an antimicrobial substances against pathogen. This research aims to isolate LAB from infant's feces and know its antimicrobial activity against *Escherichia coli* and *Candida albicans.* A total of 5 isolates *Bifidobacterium* spp. were isolated from sample. The largest inhibitory activity against *E. coli* was shown by isolate Bb3F, with inhibitory zone of 10.80 mm. While, the largest inhibition activity against *C. albicans* was shown by isolate Bb1B and Bb3F with inhibitory zone of 9.70 mm.
Keywords: antimicrobial substances, infant's feces, *Bifidobacterium*, *Candida albicans, Escherichia coli*

ISOLATION AND IDENTIFICATION LACTIC ACID BACTERIA FROM GEDI LEAVES (*Albemoschus manihot* L.)

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ABSTRACT

Indonesia is a country that has a variety of abundant types of plants such as fruits, wide range of spices, vegetables, tubers, and some of them are endemic. One of the endemic plants in Indonesia and found in North Sulawesi is Gedi (*Albemoschus manihot L.*). Gedi plants are often processed into complementary vegetables to provide a savory effect in typical foods in North Sulawesi, namely Tinutuan (also known as manado porridge). Gedi plants in addition to providing a savory effect on food can also be used as alternative medication to treat various diseases such as kidney disease, stomach ulcers, lowering cholesterol in the blood and facilitate digestion. The purpose of this research was to obtain lactid acid bacteria (LAB) from Gedi leaves. This research was conducted accordance with the qualitative descriptif of lactid acid bacteria from Gedi leaves cover up microscopic characteristic, macroscopic characteristic, and identify them based on bacterial morphology. The 32 isolates of acid-producing bacteria were successfully isolated from Gedi leaves using MRS-agar media. 32 isolates lactid acid bacteria were based on gram staining catalase, motility, spore-forming and gas production from glucose. The results of research the presence of lactic acid bacteria from Gedi leaves it can be said that bacterial morphology from 32 isolates of LAB were shaped rods and classified in the genus *Lactobacillus*.

Keywords: Gedi Leaves, Lactic Acid Bacteria, *Lactobacillus,* Plant

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Yogyakarta, August 29-31, 2019

Influence of Lactic Acid Bacteria in Commercial Probiotic on The Nitrification Process by Bacteria from Catfish Pond Sediment

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ABSTRACT

The purpose of this study was to investigate the influence of adding the probiotic containing lactic acid bacteria on the nitrification process by the bacterial isolates from a local catfish pond sediment. The study was conducted on batch system bioreactors with a working volume of 1 liter containing 100 mg/L ammonia solution and 50 grams sediment of catfish pond. This study applied concentration variations of commercial probiotics (Monodon Plus) and glucose. The variations of commercial probiotic added to the bioreactor were 5 ml/L, 10 ml/L and 15 ml/L. The variations of glucose concentration were 0 gr/L and 3,9 gr/L. Analysis of ammonia concentration was carried out by a Spectrophotometer using the Phenate method. The highest removal efficiency of ammonia was 92.35% in the bioreactor with a mixture of 15 ml/L commercial probiotic and 3.9 gr/L glucose with the fastest ammonia rate was on the third day of the experimental period. The addition of glucose in the bioreactors could increase ammonia removal by 57.39%. The result of statistical analysis indicated that variations in the concentration of commercial probiotic indicated no statistically significant difference on ammonia removal ($P > 0.05$), while variations in glucose concentration showed a statistically significant difference in ammonia removal ($P < 0.05$). Three isolates were successfully isolated from the catfish pond sediment using a selective medium for nitrifying bacteria. The result of bacterial identification showed that the three isolated bacteria were *Bacillus sp., Aeromonas salmonicida,* and *Burkholderia cepacia.*

Keywords: lactic acid bacteria, ammonia, nitrification, sediment of catfish pond, commercial probiotic.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Draft genome sequence of *Lactobacillus plantarum* Mut-7 FNCC 250, a native Indonesian strain isolated from fermented dried cassava (gatot)

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ABSTRACT

Lactobacillus plantarum is one of the most versatile species extensively used in the food industry both as bacterial starters and probiotic bacteria. *Lactobacillus plantarum* Mut7 FNCC 250 is an indigenous bacterium isolated from fermented food ("gatot"). Here we report the draft genome of strain Mut-7 FNCC 250 consists of 3,258,337 bp with 3,197 protein-coding genes in 41 contigs, 74 noncoding RNA genes, and a 44.4% G+C content. The genome contains an unprecedented proportion of regulatory genes, predominantly those likely to be involved in biosynthesis of cofactors, vitamins, protestic groups, and pigment. The genome sequence of *Lactobacillus plantarum* Mut7 FNCC 250 will greatly increase our understanding of this bacterium to be used in the food industry.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

The Effect of Dates Addition and Fermentation Time on Quality Characteristic of Coconut Water – Dates Probiotic Drink

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ABSTRACT

Dates and coconut water are known to contain rich nutrients. Both have the potential to be used as probiotic drink ingredients. The fermentation times is also known as a factor that significantly effect to quality of probiotic drink. The aims of this research are: 1) to determine the effect of dates addition on quality characteristics of coconut water-dates probiotic drink; 2) to determine the effect of fermentation time on quality characteristics of coconut water-dates probiotic drink; 3) to determine the effect of combination treatment between dates concentration and fermentation time that produce the best quality characteristics of coconut water-dates probiotic drink. Randomized Block Design was used in this resarch. The treatment were the concentration of dates (15%, 20%, 25% w/v), and fermentation time were 14 hours, 16 hours, and 18 hours, respectively. The treatment were arranged factorially and repeated three times. Physico-chemical characteristic (pH, viscosity, total acid, total solubled solid, and total sugar), microbiology characteristic (total of lactid acid bacteria), and sensory characteristic (color, aroma, taste, acidity, sweetness, and preference) were determined. The physico-chemicals data were analyzed by F test and the sensory data were analyzed by Friedman test. The result showed that increasing dates concentration up to 25% tend to increase of viscosity, total soluble solid and total sugar. In addition, increasing the fermentation times up to 18 hours tend to increase of pH, viscosity and total acid, respectively. The combination treatment of 25% dates concentration and 14 hours of fermentation time resulted the best qualitty characteristic of coconut water-dates probiotic drink.

Keywords: coconut water, dates, fermentation time, probiotic drink.

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Effect of Administration Fermented Bitter Melon Juice on Lipid Profile of Diabetic Sprague Dawley Rats

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ABSTRACT

Momordica charantia (bitter melon) contains biologically active phytochemicals including triterpenes, proteins and steroids. It has several medicial properties such as antidiabetic, antioxidative, and hypolipidemic properties. Lactic acid fermentation increase its antioxidative propeperties. The pathogenesis and progression of diabetes followed by elevated trigliseride and cholesterol levels. The aim of this study was to evaluate blood lipid profile before and after treatment. A total of 24 male Sprague-Dawley rats were used. Diabetes type 2 was induced by a single dose (60 mg/kg) of streptozotocin (STZ) and 120 mg/kg of nicotinamide, intraperitoneal injection. Following 3 days of STZ induction, the animals were randomly divided into four groups (n = 6); group 1 treated with Acarbose 40 mg/100 g feed (DM-Ac), group 2 treated with MC (DM-MC), group 3 treated with probiotic MC (DM-FMC), and group 4 as control (DM-Ctrl). Oral administration of the MC fruit extract (10 ml/kg body weight) was continued for 28 days. Groups treated with MC (non fermented and fermented) showed a significant decreased ($p < 0.05$) in cholesterol, trigliseride, and LDL compared to the DM-Ctrl group; and vice versa in HDL. Although the decrease was inferior compared to acarbose, DM-FMC intervention provided a significant improvement in the lipid profile. These results suggest that fermented bitter melon juice is a promising complementary agent for hypolipidemic.

Keywords: Bitter melon, fermented bitter melon, diabetes mellitus type 2, hypolipidemic, animal model

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Prebiotic Effects of Philippine Medicinal Plants on Mice Gut Microbiome Provide Support for Their Health Benefits

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ABSTRACT

The Philippine Department of Health promotes many indigenous plants as medicinal based on folkloric use and previous studies. These plants are mainly used as crude or partially purified decoctions thus, we hypothesized that their health benefits are partly due to the effects of the extracts on the gut microbiome, which interacts with and processes the extracts. Six groups of BALB/c mice were each given 200 mg/kg body weight of (1) *Cassia alata*, (2) *Psidium guajava*, (3) *Quisqualis indica L.*, (4) *Carmona retusa*, (5) *Peperomia pellucida*, and (6) *Mentha cordifolia* crude extracts through oral gavage for a month. Bacterial DNA was extracted from fecal samples collected before and on Day 16 and 32 of extract administration. Targeted 16S metagenomics next generation sequencing was done to determine effect of extract on gut bacteria. *C. alata,* increased Firmicutes and decreased Bacteroidetes, Verrumicrobia, Saccharibacteria in mice gut, while *P. guajava* increased Proteobacteria and decreased Bacteriodetes. This suggests that *C. alata*, with known antimicrobial activity, promotes the growth of good bacteria but inhibits harmful gut bacteria. *P. pellucida*, with anti-inflammatory activity, decreased Bacteroidetes and increased Proteobacteria but did not change Firmicutes population. *C. retusa,* also with anti-inflammatory activity, decreased Firmicutes and Proteobacteria and increased Bacteroidetes. This implies that the anti-inflammatory activity of *P. pellucida* and *C. retusa* extracts are partly due to the promotion of growth of beneficial bacteria. *Q. indica* and *M. cordifolia* extracts both increased Firmicutes and decreased Proteobacteria in mice gut, with decrease in Actinobacteria also observed in *Q. indica*-fed mice.

Keywords: prebiotic, medicinal plants, gut microbiome, next generation sequencing

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Development of Fermented Milk with *Lactobacillus paracasei* PS23 and Evaluation of Its Anti-Colitis Function

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ABSTRACT

Colitis refers to the phenomenon of inflammation of the inner of the lining colon, which may be caused by heredity, environment, diet, autoimmune, mental stress and infection. The symptoms and treatments are determined by the cause and usually focus on symptoms relief, supportive care, pain control and prevention of dehydration. Probiotics, providing gastrointestinal care and immune-regulating function, might be an alternative solution for colitis. *Lactobacillus paracasei* PS23 (PS23) has been intensively studied for its health benefits, including decelerating age-related cognitive decline and sarcopenia, and reducing the symptoms that caused by early life stress. In the present study, The PS23 strain was used to produce fermented dairy products and evaluated its anti-colitis effect. For fermentation study, we found that PS23 grew slowly and did not produce acid in milk along. Co-cultured PS23 with *Lactobacillus bulgaricus* and *streptococcus thermophilus* could significantly improve the stability of fermented samples, including syneresis. After 3-week storage, the survival rate of PS23 could maintain 90%. *In vitro* anti-colitis test indicated that the fermented product with PS23 could prevent the simulated intestinal epithelial injury with 3% dextran sulfate sodium salt reagent (DSS). Further animal study is necessary to confirm the in-vitro findings. The possible mechanisms will be also investigated through analysis of microbiome, metabolome and molecular pathway.

Keywords: Colitis; *Lactobacillus paracasei* PS23; Fermented milk; dextran sulfate sodium salt reagent.

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Cholesterol Lowering Effect of *Lactobacillus* Potential as Probiotic Isolated from Fermented Mustard

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ABSTRACT

Some strains of LAB are known as probiotics that has health benefits to reduce cholesterol. LAB can be found in various fermented foods such as fermented mustard. Ten *Lactobacillus* strains isolated from fermented mustard showed the ability in removing cholesterol in growing medium and their BSH activity varied between strains. For some strains there was no correlation between the ability to reduce cholesterol in growing medium with the BSH activity. Cholesterol reduction was due to assimilation and attachment of cholesterol on the cell wall; however mostly due to assimilation of cholesterol. Further study on 5 isolates namely *L. plantarum* 1 4C1062, L. *plantarum* 1 4C161, *L. plantarum* 1 4C962, *L. plantarum* 1 4C761, and *L. fermentum* 3B151with *L. rhamnosus* R23 as a reference revealed that the highest hypocholesterolemic activity of LAB was shown in growing cell (39.91-20.80%), followed by resting cell (18.24-12.76%), and dead cell (17.72-5.32%). Isolate 4C962 and 4C1062 showed the highest cholesterol removal activity during their growth; however the ability significantly decreased in resting and dead cells. The present finding suggest that the cholesterol lowering effect of the *Lactobacillus* isolated from mustrad should be supported by the ability of the isolates to survive in gastrointestinal track. *L. plantarum* 4C962 and 4C1062 had good survival ability in low pH and bile salt, and strong antimicrobial activity; therefore the strain could be considered as the candidate of probiotics with specific function in lowering cholesterol.

Keywords: fermented mustard, probiotic, *L. plantarum*, cholesterol lowering effect, growing cells.

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Yogyakarta, August 29-31, 2019

Potential of Ampel Bamboo Shoots (*Bambusa vulgaris*) Picle "*Lactobacillus fermentum* LLB3" and "*Lactobacillus pentosus* LLA18" As A Starter for Mozzarella Cheese And Beverage

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ABSTRACT

Isolate of *Lactobacillus fermentum* LLB3 and *Lactobacillus pentosus* LLA18 obtained from the isolation of pickled bamboo shoots used for starter making mozzarella cheese and beverage. The research was carried out using Indonesian local fruit, "duwet" fruit (*Syzygium cumini*) extract as a medium for fermentation of *Lactobacillus pentosus* LLA18. While *Lactobacillus fermentum* LLB3 is used as an inoculum for the making of mozzarella cheese with a comparison of acidulan obtained from ambarella fruit (*Spondias dulcis*) extract. This study aims to determine the potential of *Lactobacillus fermentum* LLB3 and *Lactobacillus pentosus* LLA18 isolates as a starter for making mozzarella cheese and beverage. The results showed that the content of lactic acid bacteria from "duwet" fruit extract which was fermented with *Lactobacillus pentosus* LLA18 had optimal growth in 24-hour fermentation with colonies of $2x10^8$ to $1.7x10^9$ CFU ml^{-1}. *Lactobacillus fermentum* LLB3, which was added to the process of making mozzarella cheese, showed higher yields, higher melting point, lower hardness, and longer elongation than mozzarella cheese produced with the addition of ambarella fruit extract. It can be concluded that the isolates from Ampel bamboo shoots, namely *Lactobacillus fermentum* LLB3 has the potential as a starter to make fermented beverage and *Lactobacillus pentosus* LLA18 has the potential as a starter to make mozzarella cheese.

Keywords: Bamboo shoot pickle, *Lactobacillus fermentum* LLB3, *Lactobacillus pentosus* LLA18, mozzarella cheese and beverage.

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Cytotoxic Activity of The Biofunctional Probiotic Strains *Lactobacillus plantarum* BS25 and *Pediococcus acidilactici S3* Against Colorectal Cancer Cells (HCT 116)

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ABSTRACT

Colorectal cancer is one of the leading causes of mortality worldwide and there is a need to develop chemotherapeutic agents that induce a specific cell death that is less inflammatory. This research focused on the evaluation of the cytotoxic and apoptotic potentials of cell free supernatants (CFS) from two lactic acid bacteria strains (*Lactobacillus plantarum* BS25 and *Pediococcus acidilactici* S3) isolated from Philippine traditional fermented foods against the human colorectal cancer cell line (HCT-116) via 3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. Results showed that BS25 and S3 CFS exhibited cytotoxicity on HCT-116 cells, with average IC_{50} values of 27.535% v/v and 26.090% v/v, respectively. Moreover, the cytotoxicity that was observed was further validated via morphological analysis. HCT-116 cells characteristically showed membrane blebbing, granulation, and rounding up, which are typical morphological symptoms of cell death, when treated with the bacterial CFS from both strains. TUNEL staining confirmed DNA fragmentation in HCT-116 cells upon treatment with 100% v/v CFS from BS25 and S3 within 12 hours, which is an indicator of apoptosis. These results show that the CFS from *L. plantarum* BS25 and *P. acidilactici* S3 appear to be potentially promising anti-cancer agents for adjunct therapy, which can be further purified via bioassay-guided fractionation methods.

Keywords: colorectal cancer, cell-free supernatant, cytotoxicity, *Lactobacillus plantarum* BS25, *Pediococcus acidilactici* S3, probiotics

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Establishment of An Efficient Method of Ornithine and Citrulline High-Production by A Plant-Derived Lactic Acid Bacterium, *Weissella confusa* K-28

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ABSTRACT

To practically produce many kinds of amino acids, *Corynebacterium* (*C.*) *glutamicum* is used. However, the genus *Corynebacterium* contains bacteria pathogenic to humans, such as *C. diphtheriae.* An aim of the present study is to obtain a lactic acid bacterial strain that produces ornithine and citrulline at a high level. We have successfully isolated a plant-derived lactic acid bacterium, K-28, and identified it as *Weissella* (*W.*) *confusa*. The production of ornithine and citrulline by the K-28 strain was 18 ± 1 g/L and 10 ± 2 g/L, respectively, with a 100 ± 9% conversion rate from arginine when arginine was continuously fed into a jar fermenter. Although the high-ornithine production method using *C. glutamicum* has been industrially performed, some strains are bred by mutagenesis including gene editing. The ornithine production by the wild-type of *C. glutamicum* is only 0.5 g/L, indicating that *W. confusa* K-28 is superior to *C. glutamicum* to use a probiotic microorganism.

In this study, we confirmed that *W. confusa* K-28 harbors an arginine deiminase (ADI) gene cluster composed of *wkaABDCR*. The reverse transcription PCR analysis suggested that the expression of these genes decreases with cell growth. We also show that the production of ornithine and the expression of the five genes are significantly decreased under the aerobic condition but not under the anaerobic one. The expression level of the five genes did not differ in the presence or absence of arginine, suggesting that the production of both amino acids in the K-28 strain was not induced by arginine added to medium.

Keywords: ornithine, citrulline, arginine deiminase, *Weissella confusa*, lactic acid bacteria.

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Effect of Iles-Iles (*Amorphopallus oncophyllus*) Sinbiotic Effervescent Tablet) to Decrease in Blood Sugar Levels in Hyperglicemic White Mice (*Rattus norvegicus*)

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ABSTRACT

This study aims to determine the effect of giving iles-iles synbiotic effervescent tablets to decrease blood sugar levels of hyperglycemic white rats and find out the lowest dose of effervescent tablets in decreasing blood sugar levels. This study used the Completely Randomized Design (CRD) with single factor (dose of iles-iles sinbiotic effervescent tablet) with 4 levels (dose 0.045 gram/day (½ normal dose), dose 0.09 gram/day (normal dose), dose 0.18 gram/day (2X normal dose)) and Control (without treatment dose). The analysis carried out was blood sugar levels and rat body weight. The administration of iles-iles sinbiotic effervescent tablets can reduce blood sugar levels in white mice, but does not affect changes in body weight of white mice. The biggest decrease in blood sugar levels in white rats for 1 month was obtained from the administration of a dose of 0.18 grams/day (normal 2X dose) which was as much as 49.16% The administration of effervescent tablets in the fourth week with a normal dose of 0.09 grams/200 grams of weight has been able to reduce blood sugar levels in white rats to normal levels.

Keywords: Iles-iles, synbiotic, Hyperglycemic

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Isolation, Characterisation and Anti-Breast Cancer Effects of Potential Probiotic Bacteria from Human Breast Milk

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ABSTRACT

Breast cancer is a deadly disease, impacting 2.1 million women each year, and causes the greatest number of cancer-related deaths among women with 627,000 deaths in 2018. Current standard drugs used in breast cancer treatment are mostly chemotherapeutic base most of which are accompanied with huge complicated side effects. Alternatively, biotherapeutic potentials of probiotic bacteria in cancer treatment are widely explored. In this study, potential probiotic bacteria from human breast milk was isolated, characterized and further screen as anti-breast cancer agents. One hundred bacteria were successfully isolated, and twenty isolates were further characterized using Biolog Gen III and 16S rRNA to dwell down to species levels. The results obtained from the study revealed the presence of *Staphylococcus homini, Staphylococcus lugdunensis, Enterococcus faecalis* and *Enterobacter cloacae*. Live, heat-killed cells (HKC) and the cytoplasmic fractions (CF) of *Enterococcus faecalis* and *Staphylococcus hominis* were furthered investigated their potential as anti-breast cancer agents. Non-malignant breast epithelial cell line, MCF-10A and MCF-7 cell line were treated with these components and cytotoxicity was evaluated for 24, 48 and 72 h using MTT assay. The morphological features

of the treated cells were examined by fluorescence microscopy. The stage of cell cycle arrest and apoptosis were quantified by flow cytometry. All the three forms of the bacteria caused a significant decrease in MCF-7 (up to 33.29%) cell proliferation in concentration- and time dependent manner. Morphological features of apoptosis were observed. Flow cytometry analyses suggested that about 34.60% of treated MCF-7 was undergoing apoptosis while treated MCF-10A showed no significant difference with the untreated (>90% viability). These findings highlight the potential application of these bacteria as an alternative nutraceutical with significant therapeutic indexes for breast cancer because of their non-cytotoxic effects to non-malignant mammalian epithelial cells.

Keywords: Probiotic, breast milk, apoptosis, cytotoxicity, breast cancer

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Difference of Gut Microbial Community in Indonesian Obese, Type 2 Diabetic, And Healthy Groups.

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ABSTRACT

Obesity and type 2 diabetes (T2D) mellitus have become frequently communicable diseases worldwide. Many scientific studies state that their developments are associated with disorder in gut microbial community. Indonesia is one of the countries, facing rapid increase of obese and T2D patients, where the cause and effect of its gut microbiome in these metabolic diseases are not yet to be disclosed. To this end, the 75 Indonesian volunteers were classified into three categories of normal weight (n = 35), overweight (n = 19), obese (n = 21) groups, and the two groups of T2D (n = 25) and non-T2D (n = 50). Their gut microbiota were analyzed by using 16S rRNA gene amplicon sequencing. Alpha diversity analysis showed that obesity subjects tended to show lower Shannon diversity than healthy group. Linear discriminant analysis effect size (LEfSe) showed that obese group had significantly higher relative abundance of unclassified Clostridiaceae, while normal weight group had more Ruminococcaceae, Bacteroidaceae, and the butyrate-producing Clostridiales. For the T2D, subjects administered by anti-diabetic drugs were excluded because anti-diabetic drugs are known to affect the gut microbiota. As the result, a limited group of bacteria, for example, unclassified Lachnospiraceae was significantly more abundant in the T2D group. These mentioned results suggest different mode of interaction of gut microbiota to obesity and T2D mellitus in Indonesian.

Keywords: Indonesia, obesity, diabetes, gut microbiota

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Effect of Fructooligosaccharides on The Growth and Survival of *Lactobacillus plantarum* in Model Sugar Systems

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ABSTRACT

The aim of this research effort was to investigate the effect of fructooligosaccharide (FOS), a prebiotic on the growth and viability of *Lactobacillus plantarum.* Prebiotics are substrates that is selectively utilized by host microorganisms conferring health benefits. Probiotics have been known for their ability to utilize both simple and complex saccharides for their energy production. In this study FOS concentrations (0.5%, 1%, 2%, 3%, 4%) were varied to study its effect on the growth (24 h growth curve assay) and survival (10 days survival assay) of *L. plantarum* in model glucose, fructose, lactose and sucrose systems. In glucose, and fructose model systems (3% and 4% sugar concentrations) without FOS supplementation, *L. plantarum* showed remarkable growth. However, 3% and 4% FOS supplementation in glucose and fructose showed partial inhibition where else with 0.5%, 1%, and 2% FOS supplementation showed no effect on the growth of ***L.*** *plantarum*. But in sucrose and lactose model systems (2%, 3%, 4% sugar concentration), 0.5%, 1%, 2% and 3% FOS supplementation showed growth-promoting effect on *L. plantarum.* In survival assay, 3% 4% sucrose and lactose with 3% and 4% FOS supplementation showed the best protective effect on the viability of L. *plantarum* followed by 3% and 4% fructose, glucose on day-8 and day-10. In conclusion, FOS supplementation showed different effects on the growth and survival of *L. plantarum* depending on the type and concentration of sugar and concentration of FOS used.

Keywords: fructooligosaccharide, prebiotics, probiotics

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Screening and Partial Characterization of Bacteriocin Produced by Lactic Acid Bacteria from Traditional Thai Fermented Food

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ABSTRACT

Bacteriocins are ribosomal synthesized antibacterial peptides produced by bacteria, which can kill or inhibit pathogenic strains. Bacteriocins produced by lactic acid bacteria (LAB) have gained increasing interest in the past few decades for use as the substitutes for synthetic food additives. The purposes of the present study were to isolate bacteriocin-producing LAB from Thai traditional fermented foods and study their properties. A total of 137 LAB isolates were obtained from 20 Thai fermented food samples. They were examined for bacteriocin production by agar well diffusion assay. It was found that 32 isolates showed antibacterial activity against 4 pathogenic indicator bacteria i.e. *Bacillus cereus, Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella enteritica* serovar Typhi. Among 32 LAB isolates, AHL9a displayed the highest antibacterial activity against all 4 indicator strains. Bacteriocin produced from isolate AHL9a was tested with α-amylase, lipase and proteinase K enzymes. It was found that the bacteriocin from AHL9a was hydrolyzed by proteinase K but not by α-amylase and lipase. Based on cell morphology, carbohydrate utilization and 16S rDNA analysis, isolate AHL9a was identified as *Leuconostoc mesenteroides*. As the bacteriocin from strain AHL9a could inhibit both gram positive and gram negative pathogenic bacteria, it thus has the potential application in food preservation.

Keywords: probiotic, lactic acid bacteria, bacteriocin

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Yogyakarta, August 29-31, 2019

Maternal Gut Microbiota and Probiotic Supplementation As The Potential Interface of Gut-Brain-Axis In Promoting Fetal Brain Development and Child Cognition

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Recent studies indicate human susceptibility to diseases and metabolic outcomes may be programmed during fetal developmental, and potentially linked with early life effects of gut microbiota. Establishment of intestinal microbiota during early life may be affected by dietary factors and could influence brain development. Maternal probiotic supplementation has been suggested to improve brain function through the 'gut-brain axis'. Probiotics may support infant and child cognitive development and health by protecting against pathogens, modulating the immune system, liberating beneficial nutrients, and modifying the hypothalamic–pituitary–adrenal axis. We conducted a systematic review that revealed favorable effects of maternal probiotic supplementation on particular neurodevelopmental disorders. However, evidence is limited on the effect of probiotic supplementation on fetal and early life brain development. We conducted a 10-year follow-up study investigating the effect of probiotics given during childhood on growth and developmental outcomes. The study confirmed a strain-specific effect of probiotics on neurodevelopmental outcomes and suggests strains be identified that improve cognition among children. Considering the potential benefit of probiotics on brain development and cognition, we are currently conducting a randomized trial in pregnant women of a combined intervention of probiotics and LC-PUFA compared to placebo, in the context of overall government programs for iron-folate pills,

healthy eating, and psychosocial stimulation. We will assess impact on fetal brain development as assessed by MRI, and later cognitive development using the BSID assessment at 6 months of age. Additional studies are needed to examine the effects of maternal preconceptional, pregnancy, and post partum probiotic supplementation on fetal and infant brain and cognitive development and health.

Keywords: early life, gut-brain-axis, fetal brain, child cognition, probiotics

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Divergent Effect of Rice Bran and Fermented Rice Bran on Cecal Short Chain Fatty Acid and Lactic Acid Bacteria of Colorectal Cancer Model of Mice

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ABSTRACT

Colorectal cancer is the third leading cancer worldwide. Alteration in the gut microbiota and lower level of short chain fatty acids (SCFA) are important risk factors in the development of colorectal cancer. Rice bran contains high dietary fiber and fermentation of rice bran using *Rhizopus* mold increases the dietary fiber content in fermented rice bran. However, little information is present on the impact of rice bran and fermented rice bran on the gut microbiota and SCFA level. Here, we evaluate the effect of rice bran and fermented rice bran on lactic acid bacteria and SCFA level in colorectal cancer mice model. Colorectal cancer was induced in twenty five (25) male mice aged 8 weeks by intraperitoneal injection of DMBA 20 mg/kg body weight twice a week for 3 weeks. Mice were randomly allocated into 5 groups: control, negative control, rice bran-10, rice bran-20, fermented rice bran-10, fermented rice bran-20. After 4 weeks, mice were sacrificed and cecum was collected for pH, lactic acid bacteria and SCFA analysis. Data were analyzed using ANOVA with alpha 95%. There are significant difference ($p<0.05$) among groups in the pH, log CFU of lactic acid bacteria and SCFA content. Administration of both rice bran and fermented rice bran reduced the acidity of the gut and increased the production of SCFA. Interestingly, fermented rice bran-20 has lower log CFU of lactic acid bacteria and SCFA level compared with both rice bran groups and fermented rice bran-10 albeit no statistically difference.
Rice bran and fermented rice bran have different effect on the gut microbiota of colorectal cancer.

Keywords: colorectal cancer, short chain fatty acids, lactic acid bacteria, rice bran, fermented rice bran

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Edible Mushroom Potency to Alleviate Stunting Through Gut Microbiota Modulation: A Review

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ABSTRACT

Stunting has become a major concern in Indonesia because 30.8%, almost one of the third, of under-5-years old Indonesian children in 2018 suffer for it. Children who suffer from stunting have growth faltering and less intelligence capacity. In the long term, it will affect their adult life productivity and national human resources quality. Stunting represents a nutrient requirement for children are not achieved especially in 1000 first day of life. Despite a lot of various reason, nutrient intake and infectious disease are considered as the direct cause of stunting. Recent research has explored that microbes who cohabitate human intestinal can affect their host's health and nutritive status. The composition of gut microbiota is shown different between a healthy individual and stunted individual. Hence, there is a hypothesis that stunting can be alleviated by modulating the composition of gut microbiota. Various kind of edible mushroom can be found and has been part of the diet for several Indonesian. Not only mushroom contains high dietary fiber, vitamin, and mineral, but also several mushrooms are known for its immunomodulating effect. With plentiful prebiotic potential carbohydrates, like chitin, hemicellulose, β and α-glucans, mannans, xylans, and galactans in mushroom, Mushrooms can act as a prebiotics to modulate gut microbiota, and give health benefits to the host. This paper will present several shreds of evidence that edible mushroom has potency to become a source of prebiotic, affect gut microbiota composition, and prevent stunting. It also will show any obstacles in applying edible mushroom in an attempt of combating stunting, to give future research prospect in related studies.

Keywords: stunting, gut microbiota, edible mushroom, prebiotic

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Prebiotic Effect of Plant Extracts on Growth of Probiotic Lactic Acid Bacteria and Fish

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ABSTRACT

The purpose of this study was to evaluate the effects of some plant extracts as prebiotics on the growth of probiotic *Lactobacillus paraplantarum* strain L34b-2 and their synbiotic effects in hybrid catfish. Plant samples (Chinese celery, Chayote, Dill, Curcumin, cucumber, soybean meal) were extracted with hot water prior to freeze-drying into powder, followed by subjecting to simulated acid and bile salts conditions of gastrointestinal tracts. Plant samples showed different amounts of indigestible polysaccharides (IP). The extracts from chayote and cucumber contained higher IP than those of the other plants i.e. 195.90 and 55.46 mg/g powder, respectively. The extract from each plant was determined for its ability to enhance growth of a probiotic *Lactobacillus paraplantarum* L34b-2 by cultivating the bacterium in a basal medium supplemented with each plant extract. The extract from cucumber and soybean meal was found most effectively in enhancing growth of *L. paraplantarum* L34b-2. In addition, the bacteriocin produced by strain L34b-2 against fish bacterial pathogens, *Aeromonas hydrophila* FW32 and *Streptococcus agalactiae* F3S was also enhanced by the plant extracts. Feed-trial experiments were conducted in hybrid catfish for 30 days. Growth performance of fish based on weight gain, specific growth rate and feed conversion ratio showed that fish fed bacterial probiotics and plant extract exhibited significantly highest ($p < 0.05$) growth. The obtained results demonstrate the promising synbiotic in aquaculture.

Keywords: prebiotic, probiotic, synbiotic, lactic acid bacteria

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Application of probiotics as potential absorbents for heavy metal removal in food products

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ABSTRACT

Nowadays, the pollution of the environment with heavy metals is of special concern due to their persistency in nature, toxicity and accumulation in the food chain. Various industries, including mining and metallurgy, pesticide and fertilizer industry, surface finishing industry, electroplating and electric appliance manufacturing, produce different toxic metals into the environment that are a significant threat to the public health. Studies have revealed that these elements cannot process and dispose in the human body. Therefore, they are deposited in different internal organs and cause adverse reactions. Because of their high toxicity, the reduction of heavy metals level in food products is a necessity to improve food safety. Physical, chemical and biological methods are introduced for removal of heavy metals, but physical and chemical methods have many disadvantages such as expensive equipment and incomplete metal removal. Recently, biological treatments have been more attractive. Several studies demonstrated specific strains of probiotics such as *Lactobacillus fermentum, Lactobacillus rhamnosus and Bifidobacterium longum* can remove toxic heavy metals such as Cd^{2+}, Pb^{2+} and Hg^{2+} in foods. Metal biosorption mechanisms are ion exchange process, chelation, complex formation, adsorption and microprecipitation. Binding

mechanisms are associated with the functional groups present (mainly carboxyl and phosphoryl) and S-layer proteins in probiotics cell wall that have affinity for heavy metal ions to form metal complexes. Considering the findings in different studies, some factors have an important role in toxic metals removal in food products including the strains, surface charge, contact time, temperature, presence of other cations and pH.

Keywords: Biosorption, Heavy metals, Probiotic bacteria, Toxicity

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The Effect of *Lactobacillus casei* Strain Shirota on Hemoglobin Level Among Female Adolescents

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ABSTRACT

The rapid growth and menstrual cycle every month results in female adolescent often getting anemia. This condition is exacerbated by less diverse variation of consumption patterns. The aim of this study was to analyze the effect of probiotic *Lactobacillus casei* strain Shirota on the hemoglobin level of anemia in female adolescent who suffer anemia that were supplemented with Fe + folate. The study took place at the Jabon Health Center, Jabon Sub-District in period for 4 weeks. The design of this study was a pretest posttest control group design. The treatments were divided into two groups, namely the treatment group given 1 Fe tablet + Folate per week and 1 bottle of probiotic milk Lactobacillus casei strain Shirota per day and the control group given 1 Fe tablet + Folate per week. There were two young women who dropped out after the treatment so that, only 22 young women who met the criteria for analysis. The results showed that after treatment, the hemoglobin level in the treatment group (0.7 ± 1.0 g / dl) was higher than the control group (0.1 ± 0.5 g / dl), but not significantly different. The proportion of anemia in the treatment group decreased by 36.4% and only 9.1% the proportion of anemia decreased in the control group. The conclusion of this study is that the supply of probiotic Lactobacillus casei strain Shirota can increase hemoglobin levels in the treatment group, although not significantly different.

Keywords: probiotik, *Lactobacillus casei* strain Shirota, hemoglobin, anemia, female adolescents

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Potent of Lactic Acid Bacteria Isolated from Thai Fermented Meat for Protease Production

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ABSTRACT

Protease is necessary for the healthy food production, especially for preparation of easy digestible protein. To improve the quality of fermented meat products by lactic acid bacteria, the potential probiotic *Lactobacillus plantarum* SKKL1 was selected from traditional Thai fermented meats (Nham). In this study, the ability of protease production by *L. plantarum* SKKL1 and protease properties were evaluated. To determine the protease activity, casein was used as substrate. The optimal condition of the protease was tested at pH 4 to 7 in citrate phosphate buffer and pH 7 to 8 in phosphate buffer. Moreover, the optimal temperature was evaluated from 30 ^{o}C to 50 ^{o}C. The result revealed that *L. plantarum* SKKL1 could produce protease. The maximum activity of 0.28 U/ml and the specific activity of 0.02 U/ml were obtained at 12 h of fermentation. The optimal pH and temperature were at pH 5 and 40˚C, respectively. The protease could tolerate at 40˚C for 2 h with the remaining activity of 77 %. However, it was more stable at 4˚C with the remaining activity of 79 % for 3 days. Therefore, our finding demonstrates the high potential of *L. plantarum* SKKL1 to be used as a starter culture for fermented food production and provides protease to hydrolyze protein during fermentation.

Keywords: protease, lactic acid bacteria, Lactobacillus

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Effect of Postbiotic and Inulin Supplements on Broiler Chickens

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ABSTRACT

There is an ongoing trend for reducing and prohibiting the use of antibiotics in animal feed as a result of the contamination of meat products with antibiotic residues. Thus, natural feed additives are advocated to substitute in–feed antibiotic in order to reduce food borne illnesses. The most common additives are prebiotics, probiotics, synbiotics and postbiotics (metabolic products by probiotic). Two experiments were conducted to study the effect of feeding postbiotic and inulin on performance of broiler chickens. The findings showed that the supplementation of postbiotic and inulin improved growth performance, reduced ENT and increased LAB count, prevented the shortening of villi and also had beneficial effect on meat and bone quality compared to antibiotics. The expression of IGF-I and GHR in the liver is influenced by postbiotic and inulin addition on the diet.

Keywords: postbiotics, inulin, supplementation, broiler chicken

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Genome Sequencing Through Reconstruction of Genome-Scale Metabolic Network of Lactobacillus reuteri KUB-AC5

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ABSTRACT

Lactobacillus reuteri KUB-AC5 displayed the hallmark features of probiotic properties for chicken feeds including antimicrobial production, high adhesion activity to intestinal mucus, survival at low pH and tolerance to high bile concentrations. KUB-AC5 could also protect *Salmonella* infection in chicken. The genetic characteristics of strain KUB-AC5 were explored by performing whole genome sequencing (WGS) using Illumina de novo Hiseq 4000 platform. As a result, the KUB-AC5 bacterial genome consists of a chromosome contigs with total lengths of 2,187,246 base pairs and 38.55 %GC content. Based on genome annotation, observably *L. reuteri* KUB-AC5 contained 2,196 coding sequences. The genome annotation showed that *L. reuteri* KUB-AC5 could utilize various carbon sources including maltose, sucrose, glucose, galactose, ribose and lactose. Carbohydrate utilizations were analyzed by culturing method. The growth results were confirmed genome annotation data. Focusing on annotated genes related to essential compounds for host health, thiamine)vitamin B1(, cobalamin)vitamin B12(, folate and biotin synthesis were identified. Through reconstruction process, the metabolic network of *L. reuteri* KUB-AC5 contained 724 genes, 661 metabolites, and 509 metabolic reactions. This reconstructed network can be used as a scaffold for gaining insights into cell metabolism of KUB-AC5 for further enhancing growth and vitamin overproduction. It can also serve for guiding poultry feed applications.

Keywords: Probiotics, *Lactobacillus reuteri*, Genome reconstruction, genome-scale metabolic network

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Preparation of Indigenous Lactic Acid Bacteria Starter Cultures for Large Scale Production of Fermented Milk

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ABSTRACT

Lactobacillus plantarum Dad 13, an indigenous probiotic was examined its ability to be used as a single starter culture or mixed cultures with *Streptococcus thermophilus* Dad 11 for milk fermentation. Both cultures were isolated from *dadih*, a traditional fermented buffalo milk. The purposes of this study were to produce indigenous lactic acid bacteria starter cultures using halal growth medium and evaluate their application on large scale fermented milk production. Halal medium was developed using natural compounds such as sucrose, meat peptone, mung bean extract, tomato extract and young coconut water. Meat peptone was prepared by hydrolysis of halal meat using crude bromelain. Lactic acid bacteria were grown in halal growth medium, and then harvested, frozen and freeze dried. A single freeze dried starter culture of *L. plantarum* Dad 13 and frozen mixed cultures of *L. plantarum* Dad 13 and *S. thermophilus* Dad 11 were prepared for production of fermented milk drink and yogurt respectively in industrial scale. The growth of these lactic acid bacteria in halal growth medium increased the viable cell to two log cycles (10^9 CFU/mL) for *L. plantarum* Dad 13 and one log cycle for *S. thermophilus* Dad 11 (10^8 CFU/mL) respectively. The viable cell of freeze dried *L. plantarum* Dad 13 and *S. thermophilus* Dad 11 were 7.57 x 10^{10} CFU/g and 6.35 x 10^9 CFU/g respectively. The amount of viable cells and pH of both fermented milk drink and yogurt products were relatively stable 10^7 CFU/mL and 10^8 CFU/mL respectively during cold storage for four to six weeks. Sensory characteristics of the products were comparable to the ones using commercial starter cultures. It can be concluded that these starter cultures can be applied for production of probiotic fermented milk.

Keywords: lactic acid bacteria, halal starter cultures, milk fermentation

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Antimicrobial Activity of Lactic Acid Bacteria Strains Isolated from Dadih against *Listeria monocytogenes*

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ABSTRACT

Listeria monocytogenes is an ubiquitous, intracellular pathogen which has been implicated within the past decade as the causative organism in several outbreaks of food-borne disease called listeriosis. One way to prevent food from contamination by pathogenic microorganisms is to add preservatives. Bacteriocin produced by lactic acid bacteria (LAB) is one of the natural food preservatives that is safe for human. The aims of this study were to evaluate the ability of LAB isolated from dadih in inhibiting the growth of *L. monocytogenes* and to obtain antimicrobial components that play a role in inhibiting the growth of *L. monocytogenes*. The antimicrobial sensibility of LAB to pH, heat and enzymes was carried out using the method of Mezaini et al. (2009) and crude bacteriocin production was carried out according to the method of Ogunbawo et al (2003). The results showed that supernatant from 12 LAB strains were able to inhibit the growth of *L. monocytogenes* with various inhibition zones. However, out of the 12 LAB, only 9 strains were found to have an inhibition zone of more than 4 mm. The antimicrobial compounds of 9 strains were tested and it was found that antimicrobial compounds of 3 strains derived from organic acids mainly lactic acid and 6 strains namely R-43, R-32, R-19, R-55, R-45 and R-41 derived from bacteriocin based on sensitivity to pH, heat and enzyme treatments. Crude bacteriocin derived from 6 LAB strains inhibited the growth of *L. monocytogenes,* and the highest antimicrobial activity was obtained in *Streptococcus faecalis* subsp*. liquefaciens* R-55 with an average

inhibition zone of 13.87 mm. Based on this study, it can be concluded that the bacteriocin produced by *St. faecalis subsp. liquefaciens* R-55 can be used as one of the natural preservatives for prevention from food-borne disease caused by *L. monocytogenes.*

Keywords: antimicrobial activity, lactic acid bacteria, *Listeria monocytogenes*, bacteriocin, preservative

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Zinc Enrichment of *Lactobacillus* spp. and Assessment of Its Bioavailability in Albino Wistar Rats: A Novel Biological Approach with Improved Bioavailability

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ABSTRACT

In this study, Zn enriched biomasses of *Lactobacillus fermentum* SR4 (BZ) was produced and studied for its bioavailability by *In-vivo* method and compared with organic (ZG) and inorganic Zn (ZS) when fed upto 4 weeks to male albino wistar rats. Apparent Zn Absorption (ZnAA) between Bacterial Zn (BZ) fed groups and groups fed with organic (ZG) and inorganic Zn (ZS) was significantly different. Groups fed with bacterial Zn gained significantly higher body weight in comparison to Zn depleted group and groups fed with inorganic and organic Zn. Non-significant difference was observed in femur weight gain in all groups except within BZ-30, ZS-15 and ZD. Serum Metallothionein levels were significantly higher in BZ-30 compared to all other groups. Significant difference was seen in the antioxidative enzymes (Catalase and SOD) in liver between BZ-30 and ZD groups. Significantly lower level of Zn in hair was observed in ZD group in comparison to all other groups. Liver Zn level was significantly higher in BZ-30 as compared to all other groups. Femur bone Zn level of ZD group was significantly lower compared to all groups, however, there was non-significant difference in other groups viz., basal, ZS-30, ZG-15, ZG-30 and BZ-15, BZ-30. Serum Zn level was significantly different in each group except ZS-30 and ZG-15. The present investigation establishes the potential of human origin indigenous lactobacilli of accumulating significant amount of Zn in its biomass and ability of delivering the same mineral in a highly bioavailable form. This potent culture can be explored for the development of Zn enriched functional dairy foods and as well as a Nutraceuticals.

Keywords: Bioavailability, Zinc, *Lactobacilli*, Deficiency, Antioxidative Enzymes

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Lactobacillus paracasei C2I12 Treatment of Meat from Old Philippine Native Cows

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ABSTRACT

Meat from old animals are generally of poor quality because of toughness and low juiciness. *Lactobacillus paracasei* C2I12, a proteolytic Lactic Acid Bacteria isolated from *Nipa fruticans* inflorescence was used to treat meat from old Philippine native cows. Rib roasts were divided into three parts and randomly assigned to the following treatments: untreated (U), milk treated (MT) and *Lb. paracasei* C2I12 treated (LT) samples. Sensory characteristics of the meat were evaluated after 7 and 14 d of storage at 2-4°C through quality scoring. Percent frequency of panel scores show that the Untreated was better in terms of sensory characteristics when aged for 7 d. However, when aged for 14 d, LT- treated beef had better sensory traits as indicated by absence of off flavor, improved color and flavor. In another experiment, *Longissimus dorsi* muscles were evaluated for the selected meat characteristics after 0 d, 7 d and 14 d aging. Results show that *Lb. paracasei* C2I12 was able to improve the quality of beef from old cows especially in terms of shear-force, an objective measure of tenderness. Hence, this treatment can also be used to add value to tough beef cuts. Further studies is recommended to optimize the use of this organism in shortening the aging period of beef for economic reasons.

Keywords: aging, beef, old cows, lactic acid bacteria

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Microbial Diversity In Traditional Kefir Using Culture-Independent Methods

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ABSTRACT

Kefir is an acidic-alcoholic fermented milk product with little acidic taste and creamy consistency that was originated in many places. Kefir can be produced by fermenting milk with many kind of starter such as commercial freeze-dried kefir, starter cultures, traditional kefir grains, and the product that remains after the removal of kefir grains. In this research we using a traditional kefir grains to producing kefir and tried to identified microorganisms associated in traditional kefir. Therefore we known kefir grains have a complex composition of microbial species such as the predominance of lactic acid bacteria, acetic bacteria, yeasts, and fungi. But, in any case research the researcher found difference microorganisms identified in the identical sample. There are microorganisms associated with traditional kefir were investigated using a phenotypic method. Conventional culturing was performed and a total of 118 isolates were obtained from sample. Our identification isolates from traditional kefir that we have, we found Lactic acid bacteria (82,20%) were the major isolated group identified, and yeasts (17,80%). Lactic acid bacteria isolated group identified are *Lactobacillus paracasei* (39 isolates), *Lactobacillus parabuhneri* (21 isolates), *Lactobacillus casei* (12 isolates), *Lactobacillus kefiri* (11 isolates), *Lactococcus lactis* (14 isolates), and yeast group identified is *Saccharomyces cerevisiae* (21 isolates) were the microbial species isolated.

Keywords: Lactic acid bacteria, Saccharomyces, traditional kefir

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Yogyakarta, August 29-31, 2019

Utilization of Lesser Yam (*Dioscorea esculenta l.*) Flour as Prebiotic in Yogurt to Total Lactic Acid Bacteria (Lab), Sugar Reduction, and Organoleptic Properties

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ABSTRACT

This study aims to determine the effect of the concentration the addition of lesser yam as prebiotic to total Lactic Acid Bacteria (LAB), reducing sugar content, crude fiber, viscosity, and organoleptic properties of yogurt with a combination of three bacteria (*Streptococcus thermophillus,Lactobacillus bulgaricus*, and *Lactobacillus acidophillus*). The design of this study used a completely randomized design (CRD) with 4 treatments and 5 replications with variations in the addition of lesser yam tuber, namely T1 with a concentration of 0%, T2 with a concentration of 2%, T3 with a concentration of 4% and T4 with a concentration of 6%. The raw materials used are pasteurized fresh cow's milk, lesser yam tuber flour, and yogurt starter. The results showed that the addition of different lesser yam tuber flour had a significant effect (P <0.05) on total LAB, sugar reduction, crude fiber, viscocity, and organoleptic properties of yogurt. The ideal treatment for the addition of lesser yam tuber flour is the concentration of 2% lesser yam tuber, which produces a total LAB is 9.2 x 10^9, a sugar reduction is 0.653 mg/mL,crude fiber is 1.3%, 82.25 cPs, and organoleptic properties had sour taste and viscosity is rather thick which the most preferred.

Keywords: yoghurt, prebiotic, Lactic acid bacteria, yam tuber flour

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Development and Validation of A Novel *In Vitro* Probiotic Screening System

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ABSTRACT

Numerous probiotics are being widely consumed to boost the individual's microbiome and/or to restore the gut ecosystem. However, recent publications revealed individual responses to probiotic supplements and varied changes in the gut microbiota (Kolmeder et al., 2016, Zmora et al., 2018). These and other related recent findings have paved the way for personalized probiotics, but current personalized products are solely based on the fecal microbiome analysis while overlooking gut microbe-probiotic ecological interaction. Therefore, we developed a novel biomarker-based *in vitro* probiotic screening system, the "Probiotic Meta-Analytical Screening System" (PMAS). This system is mimicking the proximal colon region in terms of medium, pH, anaerobic conditions, and temperature.

In the PMAS system, an individual's fecal sample is homogenized with medium and anaerobically incubated together with application of several different probiotic candidates, respectively; the total working volume is 600 µL. After 24 h of incubation, the proper probiotic is decided for each person based on levels of short-chain fatty acids and microbiome changes compared to negative control samples. We also validated the PMAS system with 24 different hypercholesterolemic patients. Clinically, 50% of the patients showed no SCFAs increase after the consumption of *Lactobacillus rhamnosus BFE5264* (formerly showing potential for alleviating hypercholesterolemia in a murine model) at a dose of $1x10^9$ CFU for 1 month while the other 50% of the patients did. This trend was also observed in the PMAS system with the same subjects and the same strain. In this way, a high throughput and cost-effective personalized probiotic screening system could be developed and clinically validated.

Keywords: simplified colonic fermentation model, personalized probiotics, *in vitro* screening system

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Evaluating The Correlation Between Microbiome And Metabolome Associated with The Mastitis in Holstein Dairy Cows

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ABSTRACT

Dairy cow mastitis is one of the major economic challenge to the dairy industry worldwide. Typically, mastitis is an inflammation of the mammary gland, but not limited in bacterial infection. Previous studies have shown that the composition of microbiome and metabolite was one of the important factors affecting the inflammatory response of the host. Thus, the purpose of this study was to determine the correlation between microbiota and metabolites associated with the mastitis in Holstein dairy cows. Thirty dairy cows were selected and separated into healthy and mastitis groups according to veterinary diagnosis, milk somatic cells, and milk enzyme results. The ruminal microbiota and metabolites were determined using next generation sequencing (NGS) and high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS), respectively. NGS results indicated that the community richness and diversity indices were not different between groups. Further examination by the partial least squares discriminant analysis (PLS-DA) showed a clear differentiation of healthy and mastitis group. We then evaluated the compositions of ruminal microbiota and identified the bacterial biomarkers using linear discriminant analysis effect size (LEfSe) analysis. Results indicated that biomarkers including seventeen genus level and three species level were identified in the healthy and mastitis group. For ruminal metabolome, 1181 metabolites were significant difference between groups including 65 identified metabolites (VIP > 1, p < 0.05.) by LC-MS/MS and used Mann-Whitney U Test. Additionally, the relationship between ruminal microbiota and metabolites was also evaluated by Spearman's correlation coefficient. The finding could provide a better understanding for the dairy cow mastitis.

Keywords: Holstein dairy cow, mastitis, ruminal metabolome, ruminal microbiota

ABSTRACTS OF POSTER PRESENTERS

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Contribution of Lactic Acid Bacteria to Vitamin B_{12} Content in Indonesian Tempeh

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ABSTRACT

Lactic acid bacteria (LAB) traditionally known as auxotrophic for Vitamin B_{12} (cobalamin). During tempeh production especially soaking process the bacterial population was dominated by 10^6 - 10^9 CFU/g of LAB. Vitamin B_{12} content in Indonesian tempeh generally correlated with the presence of *Enterobacteriaceae* such as *Klebsiella pneumoniae* and *Citrobacter freundii* that known have *de novo* cobalamin biosynthesis pathway. We investigated vitamin B_{12} production potency 11 LAB isolated from tempeh sample collected at the traditional market in Malang (MK1, MK3); Yogyakarta (HW2); Sleman (HV1, HV2 HX3); Magelang (SM2, SM3); Ambarawa (PA3); Bogor (PAPI, PADAI) Indonesia by employing vitamin B_{12} assay medium (HIMEDIA). Isolate PAPI, HX3 dan HV2 failed growth on vitamin B_{12} assay medium which Vitamin B_{12} free medium and containing all other vitamins and nutrients essential for growth. Molecular identification of isolate that can survive on Vitamin B_{12} assay medium employing 16s rRNA gene showed; MK1, MK3, SM2, SM3, PA3, PADAI similar with *Lactobacillus fermentum* and HW2, HVI with *L. plantarum*. Recently some LAB such as *L. plantarum* LZ95, *L. plantarum* CY2, *L. rossiae* and *L. reuteri* CRL1098 identified as bacteria strain known to encode a complete *de novo* biosynthetic pathway of vitamin B_{12}. This preliminary study showed the presence LAB during production might contributed to vitamin B_{12} content in tempeh.

Keywords: Indonesian tempeh, vitamin B_{12}, lactic acid bacteria

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Bacterial Population Dinamics of Natural Fermentation of Sumbawa Mare's Milk Using Metagenomic Approach and Physicochemical Aspects

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ABSTRACT

Naturally fermented Sumbawa mare's milk is a mare's milk product that has been fermented naturally involving indigenous microbes. This study aimed to assess the changing of bacterial density and the physicochemical aspects during natural fermentation of Sumbawa mare's milk, and to evaluate the dynamics of bacterial population during the natural fermentation using metagenomic approach. Mare's milk samples obtained from Regency of Dompu, Province of West Nusa Tenggara were naturally fermented at room temperature for 60 days. Fermented milk samples were collected on the day of 0, 7, 15, 30 and 60 for further analysis, such as bacterial density enumeration (lactic acid bacteria and aerobic bacteria), nutrition content (physicochemical aspects), physical properties of the milk, and total DNA isolation. The total DNA samples obtained were analyzed using next generation sequencing as part of metagenomic study. The research results showed the density of lactic acid bacteria on MRS agar and M17 agar decreased along with fermentation periods. Meanwhile, the density of aerobic bacteria on PCA media was relatively fluctuated. The physicochemical factors of mare's milk also changed during fermentation periods. The carbohydrate content and total sugar decreased along with the decreasing of pH value. Moreover, the lipid content increased, and the protein content fluctuated during the fermentation periods. Mare milk physical properties was changing, such as the whey color turn to yellow, the more acidic odor, and the forming of gases. The metagenomic analysis results showed the bacterial diversity index of sample on each fermentation period categorized as low indicated by the dominance of *Lactobacillus helveticus* until the end of fermentation. *Lactobacillus helveticus* as a member of LAB did not grow on isolation media at the end of fermentation period. Detection of uncultivable bacteria using metagenomics approach fulfilling the limited information on the bacterial composition of fermented Sumbawa mare's milk products.

Keywords: natural fermentation, bacterial diversity, *next generation sequencing*, Sumbawa mare's milk

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Viability Local Probiotics on Yogurt with Supplementation of Purple Sweet Potatoes to Prevention of Diarrhea in Experimental Rats

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ABSTRACT

Probiotic bacteria have health effects for their hosts, including overcoming diarrhea caused by pathogenic *Escherchia coli* (EPEC). The purpose of this study was to study the effectiveness of local probiotics in yogurt with supplementation purple sweet potato extract to prevent diarrhea caused by EPEC in albino (*Rattus novergicus*) rats *Spraque dawley* strains. The study was carried out in a Completely Randomized Design for 21 days using 20 male rats from the *Spraque dawley* albino (*Rattus novergicus*) which were divided into 5 experimental groups. Group 1 was treated yogurt with supplementation of purple sweet potato extract, commercial culture, group 2 was treated yogurt with supplementation of purple sweet potato extract, commercial culture mixture and *Lactobacillus plantarum* Dad 13 as probiotic, group 3 was treated yogurt supplementation with purple sweet potato extract, commercial culture mixture and *Lactobacillus acidophillus* SNP-2 as probiotic orally, 10^9 CFU / ml each and EPEC 10^8 CFU / ml infected for 7 days in the middle of the treatment. The fourth group was positive control treated EPEC for 7 days in the middle of the study, while group 5 was a negative control which was not treated probiotics nor EPEC. The results showed that groups of rats treated with yogurt with supplementation of purple sweet potato and commercial culture probiotic mixtures had appearance of weight growth and PER value was better than the positive control group. Groups of rats treated with yogurt supplemented with purple sweet potato extract, commercial culture mixture and probiotics in both *Lactobacillus plantarum* Dad 13 and *Lactobacillus acidophillus* SNP-2 each were able to reduce EPEC population in 1 siclus log

Keywords: Yogurt, purple sweet potato extract, local probiotics, diarrhea prevention.

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The Effect of Celery Yogurt Intervention on The Lipid Profile of Wistar Rat Fed High Oxidized Fat

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ABSTRACT

Dyslipidemia is one of the causes of coronary heart disease. This disturbance was triggered by the habit of eating fried foods which was fried in improperly frying oil. Celery yogurt may can be prevented this condition by improving lipid profile. The objective of this study is to determine the effect of celery extract yogurt on LDL levels and HDL levels of Wistar rats fed high oxidized fatty foods. This study is a quasi-experimental research with pre-post control group design. The subjects were 30 male Wistar rats of 8 weeks old with average weight was 173.80 ±6.18 grams; divided into 6 treatment groups. The K1 group (normal control) was fed standard feed and mineral water. The K2 group (negative control) was given high oxidized fatty foods and mineral water. The K3 group was given standard feed, mineral water, and ordinary yogurt. The K4 group was given standard feed, mineral water, and celery yogurt. The K5 group was given high oxidized fatty foods, mineral water, and ordinary yogurt. The K6 group was given high oxidized fatty foods, mineral water, and celery yogurt. Yogurt intervention was carried out for two months through the sonde. Lipid profile was measured before and after the intervention; by using CHOD-PAP precipitation method. The data obtained were analyzed using paired t-test and post-hoc anova test on 5% significance degree. The result indicate that intervention of celery yogurt caused significantly decreases in Triglyceride, Total Cholesterol, and LDL levels; and significantly increases in HDL levels ($p < 0.05$). Provision of celery yogurt can maintain a lipid profile, although consuming foods with a high content of oxidized fat.

Keywords: celery yogurt, lipid profile, high oxidized fat

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Novel function of dual fermented rice bran to improve metabolic-related diseases in SHRSP

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ABSTRACT

Fermented rice bran (FRB) is more enriched substances than raw rice bran. Our previous studies reported that single FRB with Rhizopus oligosporus enhances total phenolic content, antioxidant activity, and improving hypertension in stroke-prone spontaneously hypertensive rats (SHRSP). The objective of this study was to evaluate the physiology properties of dual FRB and the mechanisms of action for its application as an ingredient. FRB was prepared by dual fermentation using fungi and lactic acid bacteria. The first step, rice bran fermented with Aspergillus kawachii then the second step, rice bran fermented with a mixture of lactic acid bacteria (Lactobacillus brevis, Lactobacillus rhamnosus, and Enterococcus faecium). Male SHRSP was single oral administered of FRB and distilled water as the control (C) via a gastric tube. The hypotensive effect of FRB was observed starting from 1 h until 6 h after administration. Furthermore, chronic administration of FRB significantly reduced blood pressure and increased serum angiotensin-converting enzyme inhibitory activity. FRB supplementation improved glucose and serum insulin levels. These results suggest that dual fermentation of rice bran improve metabolic-related diseases in SHRSP and one of candidate process to enhance functional properties of rice bran.

Keywords: fermented rice bran, fungi, glucose metabolism, lactic acid bacteria, metabolic diseases.

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Detection of Putative Genes Encoding Bile Salt Hydrolase (*Bsh*) In Probiotic Strains Isolated From Indonesia Local Cultures.

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ABSTRACT

Lactic acid bacteria as a probiotic has ability to survive in growth medium which contains bile salt such as on gastrointestinal tract. Bile salt hydrolase are able to deconjugated bile salt that it make less toxic for growth bacteria. *Lactobacillus casei* strain AP and *Lactobacillus casei* strain AG were isolated from human gastrointestinal tract while *L. paracasei* strain M104 and *Pedicoccus pentosaceus* strain M103 were isolated from goat milk, they have potential as probiotics. The objective of this study was to detect *bsh* gene responsible to express bile salt hydrolase enzyme. The gene detection was perfomed by parsial gene amplification in conserve location of conjugated bile salt hydrolase (*bsh*) gene from *L. casei* str. Zhang. Primer designed has product amplification target to1016 bp. Sequence DNA analysis were aligned with references by Blastn and Blastx accessed on NCBI database. The result of amplification in *L. casei* strain AP, *L. casei* strain AG, *L. paracasei* strain M104 and *P.pentosaceus* strain M103 have length DNA sequence 989, 976, 864 and 1006 bp respectively. Similarity of these amplicon compared to *bsh* gene from L. casei str. Zhang are 99%; 100%; 98.15% and 99% respectively. All of amplicons were indentified as protein linier amida C-N Hydrolase by sequence identitty *L. casei* strain AP (100%), *L. casei* strain AG (100%), *L. paracasei* strain M104 (98.46%) and *P.pentosaceus* strain M103 (98,14%). These four strains have a partial DNA sequence as a putative *bsh* gene.

Keywords: probiotic, *bsh* gene, amplification, and bilesalt

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Isolation of Proteolytic Lactic Acid Bacteria From Indigenous *Chao* Product

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ABSTRACT

Chao is a traditional fermented fish products from Pangkajene and Kepulauan Regency, South Sulawesi of Indonesia. Lactic acid bacteria (BAL) proteolytic is one of the bacteria that plays a role during the chao fermentation process. The aim of the study was to isolate, select and identify proteolytic lactic acid bacteria (LAB) from *chao.* A total of was obtained from two producers in the Pangkajene and Kepulauan Regency, South Sulawesi. Namely, Fishery Product Processing Group, Katojowa, in Labakkang District (Fin code) and Home Industry in Bungoro District (Ags code). Sampling is carried out every 2 days of fermentation for 16 days and starts from the beginning of fermentation. The sample was packed in a cooler box that was given ice cubes and transported to the Microbiology Laboratory, Center for Food and Nutrition Studies, Center for Inter University, Gadjah Mada University of Yogyakarta to isolate the LAB content. LAB was isolated by poured plate method. Purification isolates was performed using streak method. Proteolytic LAB were selected using agar skim milk media. Protease activity of LAB was determined based on the amount of tyrosine released in unit/mL. The result showed that a total of 60 isolates were obtained from *chao,* 57% of them are cocci-shape and other are rod-shape. Fifteen isolates were proteolytic LAB. Their R values were 2.11 to 3.39. All of them have protease activity between 0.267 to 0.304 U/mL. LAB can be isolated from *Chao* products every day during fermentation.

Keywords: *chao*, fish fermented, lactic acid bacteria, proteolytic LAB

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Antimicrobial Activity of Lactic Acid Bacteria Isolated From *Ronto*, A Traditional Fermented Shrimp Paste From South Kalimantan

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ABSTRACT

The aim of this research was to study the antimicrobial activity of lactic acid bacteria (LAB) isolated from fermented shrimp *(ronto)*. Cell-free supernatant of 17 lactic acid bacteria were evaluated their anti-microbial activity against spoilage and pathogenic bacteria using agar-well diffusion assay method. The anti-microbial activity of various concentration of lactic acid, acetic acid and salt (NaCl) against spoilage and pathogenic bacteria were also tested. The cell-free supernatant of all LAB isolates inhibited the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*, but did not inhibit *Bacillus subtilis* dan *Salmonella typhimurium*. However, there were not any inhibition activities of neutralized cell-free supernatant against all bacteria tested. Lactic acid and acetic acid showed significant inhibitory effect at concentration of 0.5% except for *S. typimurium*. *Salmonella typimurium* could not be inhibited by both 0.5% acetic acid and lactic acid. The higher the concentrations of acetic acid and lactic acid the greater the inhibition zone. Acetic acid showed greater inhibitory effect than lactic acid to all microorganisms tested. Salt concentration of 10% or higher significantly inhibited the growth of spoilage and pathogenic bacteria. It can be concluded that the inhibitory activities of LAB isolated from ronto were mainly due to the organic acids produced by LAB. Salt also play an importnt role in the inhibition of the growth of spoilage and pathogenic bacteria.

Keywords*: anti-microbial activity, ronto, lactic acid bacteria, organic acids, salt*

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Production Synbiotic Drinks From Sweet Potatoes (*Ipomoea batatas*) with Probiotics *Lactobacillus plantarum* B1765

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ABSTRACT

In recent years there has been an increase in interest in developing new functional food products. One functional food product that develops is synbiotics. Synbiotic is a combination of prebiotics and probiotics. In a previous study purple sweet potato (Ipomoea batatas) was included in food containing prebiotic because it contains fructooligosaccharides (FOS), inulin and raffinose. *Lactobacillus plantarum B1765* is a probiotic isolate from used fish milk (Chanos chanos). The combination of purple sweet potato extract (ipomoea batatas) with probiotic *Lactobacillus plantarum B1765* has the potential to be developed as a synbiotic combination. The purpose of this study was to determine the time of fermentation of synbiotic drinks purple sweet This study used a post test only control group design method with a variation of the treatment of fermentation time 12, 24, 36 and 48 hours. This research is divided into 2 stages, the first stage is organoleptic test (taste and color), then followed by the second stage, namely the test of pH value and the number of lactic acid bacteria (LAB). The results showed that at a variation of 36 hours fermentation time the highest taste value was obtained at 3.4 with a pH value of 3.40 and the amount of BAL 3.264×10^6 cfu / mL. The highest color value was obtained at a variation of 48 hours fermentation time with a pH value of 3.37 and the amount of BAL $2,938 \times 10^6$ cfu / mL. These results have met the minimum standards of the amount of probiotics in drinks from the International Dairy Federation (IDF).

Keywords: Synbiotic, Purple sweet potato, *Lactobacillus plantarum* B1765, Probiotic

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The Effects of Consumption of Indigenous Probiotic Powder Containing *Lactobacillus plantarum* Dad-13 on The Body Mass Index and The Population of *Prevotella*, *Bacteroides fragilis* and *Clostridium coccoides* on Malnourished Children of Belanting Elementary School, East Lombok

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ABSTRACT

Malnutrition can be caused by insufficient food intake, including deficiencies in macronutrients (protein, total energy) or in micronutrients (vitamins, or metals such as zinc and selenium) and pathogenic bacterial infections. The aim of this study was to determine the effects of the consumption of probiotic powder *Lactobacillus plantarum* Dad-13 on the Body Mass Index (BMI) and the population of *Prevotella, Bacteroides fragilis* and *Clostridium coccoides* in malnourished children of East Lombok, Indonesia.

This study, involving 40 children aged 10-12 years and with a BMI/age ≤ -2 SD, used *a Randomized Double-Blind Placebo-Controlled* design. The children were divided into 2 groups, one of which would consume a probiotic powder product (consisting of *L. plantarum* Dad-13) as much as 1.18 x 10^9 CFU/gram. While the other consumed 1 gram of skimmed milk, once a day for 60 days. Research data included measurements of body weight, height and analysis of gut microbiota (*Prevotella, B. fragilis* and *C. coccoides*) with *real-time polymerase chain reaction* (real-time PCR) methods from feces samples taken before and after consumption.

The results showed a significant increase in the BMI of the probiotic group by 0.89 ± 0.36 from 14.09 ± 1.31 to 14.98 ± 1.30 (p = 0.00) while in the placebo group there was no significant change in BMI before or after the

study (14.64 ± 1.12 to 14.73 ± 0.89; p = 0.56). Statistically there were no significant changes in the numbers of *Prevotella, B. fragilis* and *C. coccoides* in the feces samples. The conclusion of this study is that the consumption of probiotic powder *L. plantarum* Dad-13 for 60 days can significantly increase the body mass index (BMI) of malnourished children, yet statistically have no significant effect on the numbers of *Prevotella, B. fragilis* and *C. coccoides* in the feces of malnourished children in Belanting, East Lombok. However, the prevalence of these bacteria increased 100%, 94.44% and 61.11% respectively in the volunteers after consumption of the probiotics powder.

Keywords: BMI, Gut microbiota, *L. plantarum* Dad-13, Malnourished

10th Asian Conference on Lactic Acid Bacteria
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The Effects of Consuming Capsule Containing *Lactococcus lactis* subsp. *cremoris* FC to Overcome Constipation of Indonesian Women

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ABSTRACT

Constipation is one of disorders on digestive system with the symptoms such as difficulty of defecation, hard and big feces, stomachache, discomfort on stomach area, which occur for ≤3 days every week. *Lactococcus lactis* subsp. *cremoris* FC is one of probiotic bacteria which has been known its beneficial health effects to overcome the constipation among adults. The objective of this single-blind study was to evaluate the consumption effects of *Lc cremoris FC* on 40 constipated women subjects, aged at 20-50 years that suffered constipation in Indonesia for 70 days. Each subjects would consume placebo (2 weeks) continued by *Lc*. *cremoris* FC in low dose tablet (≥ 2 X 10^7 cfu/capsule)(2 weeks), and high dose tablet (≥ 4 X 10^7 cfu/capsule) for the last 2 weeks. During this research, subjects have to fill the daily record, fecal characterization, and feeling after defecation.

No significant difference was found in defecation frequency. Stool volume measured by the Bristol Stool Form Scale was significantly better in the high dose probiotic phase than in the observation phase (2.56±0.70 vs. 2.47±1.47, $p = 0.044$). However, the levels of other fecal characteristic (consistency, odor, color, and feel after) did not differ significantly between the phases. In this study, we found that the water and fiber percetage fulfillment were low in each phase (<50%), which might be the cause of the constipations

Probiotics significantly ameliorated stool volume in patients with chronic constipation. In addition, the beneficial effect of *Lactococcus lactis* subsp. *cremoris* FC on stool consistency remained after the probiotic supplementation was discontinued. The mechanism whereby probiotics benefit patients with chronic constipation should be clarified in further studies.

Keywords: constipation, probiotics, *Lactococcus lactis* subsp. *cremoris* FC

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Isolation of Probiotic Lactic Acid Bacteria with Bile Salt Hydrolase and Uricase Activity from Fermented Spider Plant

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ABSTRACT

Hyperlipidemia and hyperuricemia are commonly found in elderly people and many studies reveal that probiotic bacteria can reduce lipid and uric acid in serum. Probiotic lactic acid bacteria can be isolated from various sources of fermented vegetables such as kimchi and sauerkraut. In Asian country especially South-East Asia, spider plant (*Cleome gynandra* L.), can be fermented as a kind of pickle, which be called Pak Sien Dong in Thailand. A few unofficial reports provided information that probiotic lactic acid bacteria are able to be isolated from Pak Sien Dong leading to the aim of this study which focus on isolation of new lactic acid bacteria with bile salt hydrolase and uricase activity. The fermented spider plants in local market of Thailand were collected and performed lactic acid bacteria isolation. From result showed that two new strains of lactic acid bacteria have ability to produce bile salt hydrolase, uricase and also have potential to be probiotic bacteria with good *in vitro* colonizing properties, which are hydrophobicity, gastric fluid tolerance and bile salt tolerance. The new strains were identified as *Lactobacillus plantarum* and *Pediococcus pentosaceus*. From the study could be suggested that novel strains of lactic acid bacteria from fermented spider plant or Pak Sien Dong in Thailand possible to be further used as probiotic bacteria for hyperlipidemia and hyperuricemia treating.

Keywords: Lactic acid bacteria, bile salt hydrolase, uricase, spider plant.

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Yogyakarta, August 29-31, 2019

Antioxidant Activities and Viability of Lactic Acid Bacteria in Yogurt Made from Buffalo Milk with Addition of Blewah (*Cucumis melo* L var. *reticulatus* Naudin) Juice

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ABSTRACT

Blewah (*Cucumis melo*) has been known to have antioxidant activity. The results of Islamil et al. (2010) revealed that the flesh of blewah had hydroxyl radical trapping activity of 67.19 ± 8.90 (g DMSOE/g extract) with the ability to trap DPPH free radicals at 11.9 ± 1.00 (IC50 (mg / mL)) with a total phenolic compound content of 1.68 ± 0.14 (mg GAE/g extract). This study began with the making of blewah juice (*Cucumis melo*) as well as inoculation and multiplication of yogurt starters to then make the process of making yogurt with the addition of blewah juice (*Cucumis melo*) as much as 10% of the total ingredients used. The purpose of this study was to determine the effect of the addition of blewah juice (*Cucumis melo* L var. Reticulatus Naudin) on the viability of lactic acid bacteria and the antioxidant activity of yogurt produced and to obtain the best storage time of yogurt based on the viability of lactic acid bacteria and antioxidant activity produced. The results indicated that the phenolic compounds contained in yogurt added with blewah juice did not contain components that had specific bactericidal activity on the culture starter. It was seen that on the first day of refrigerator storage (4^{o}C), the number of lactic acid bacteria remained at 10^8 log CFU/g and only showed a decrease in the number of lactic acid bacteria to 10^7 log CFU/g until the end of the storage process in the refrigerator (4^{o}C). Antioxidant activity of yogurt with the addition of blewah juice occurred at the 7th day of storage in the refrigerator. It was assumed that on the 7th day, lactic acid bacteria were able to metabolize phenolic compounds through hydrolysis of phenolic glycosides to aglycone so as to increase antioxidant activity until the 7th day of storage in the refrigerator.

Keywords: Yogurt, blewah (*Cucumis melo*), lactic acid bacteria, antioxidant activities

10th Asian Conference on Lactic Acid Bacteria
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Fermentation Effectivity of Milk Supplemented with *Ficus hispida* Linn.f. Leaves Extract by *Lactococcus lactis* sbsp. *lactis*

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ABSTRACT

Herbal extract supplementation could be applied for quality improvement of fermented milk. *Ficus hispida* leaf is a well-known traditional medicine from Southeast Asia because it's rich phenolic compound and antioxidant activity. This study aims to determined the effect of *Ficus hispida* leaf extract supplementation on *Lactococcus lactis* sbsp. *lactis* fermenting effectivity, total phenolic content of milk and physical characteristics (total solid and moisture content) during 24 hours milk fermentation. This research was true experimental with completely randomized factors, with the addition of 0%; 0.1%; and 0.4% *Ficus hispida* leaf distilled water extract to the mixture of cow milk, skimmed milk, sucrose, gelatin and *Lactococcus lactis* sbsp. *lactis* starter. In each six hours interval, total plate count, pH value, Total Titrable Acidity value, total phenolic content, total solid and moisture content were measured. The result obtained showed that supplementation of *Ficus hispida* left extract significantly influenced to increased viability of starter culture, total phenolic content, Total Titrable Acidity value and decreased pH value. Supplementation of Ficus *hispida* leaf extract didn't significantly influenced to change total solid and moisture content. Fermented milk with 0.4% *Ficus hispida* leaf extract had the highest viability (8.907 ± 0.221) and total phenolic content (39.033 ± 1.698).

Keywords: Fermented milk; *Ficus hispida* Linn.f. leaf extract; *Lactococcus lactis* sbsp. *lactis*; Probiotic.

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Effect of Probiotic Additive SYNSEA™ FeedAd on Growth Performance, Antimicrobial Ability and Gut Morphology of White Shrimp in Field Trial

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ABSTRACT

Pacific white shrimp (*Litopenaeus vannamei*) is economically important species of aquaculture in Southeast Asia and Taiwan. The aim of this study was to investigate the effect of feeding probiotic on growth performance, antimicrobial ability and gut morphology of white shrimp at commercial aquaculture farms. Total of three hundred thousand larval stage shrimps (average weight of 3.4mg) were randomly divide into three groups and adjusted to the same density. These three groups were fed with basal diet (control), basal diet plus multi-strain probiotic SYNSEA™ FeedAd (containing *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, and *Bacillus subtilis)* and basal diet plus heat-killed SYNSEA™ at 10^6CFU/g feed, respectively. The results showed that, live and heat-killed probiotic could significantly enhance the feed conversion rate (FCR), specific growth rate (SGR) and survival rate ($P < 0.05$). Gram-negative bacterial flora, such as *Vibrio spp*. and Coliform counts in the shrimp midgut were significantly decreased in both live and heat-killed probiotic-fed group after 12 and 16 wks. ($P < 0.05$), and the lactic acid bacteria counts in probiotic groups were found significantly higher than the control group ($P < 0.05$). However, only live SYNSEA™ FeedAd could significantly increase the gut villi height of white shrimp after fed 12 weeks compared with control group. The results suggest that, SYNSEA™ FeedAd can be a dietary probiotic no matter in live or heat-killed form for white shrimp in promoting growth performance and protecting the gut health of shrimp to avoid the opportunistic infection.

Keywords: Antimicrobial ability, Growth performance, *Litopenaeus vannamei*, Probiotic

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Organoleptic Assessment, Cholesterol Level and Total Colony of *Lactobacillus acidophilus* In Piskebal (Pisang Kepok Bakteri Asam Laktat)

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ABSTRACT

Background: Synbiotics are a combination of probiotics and prebiotics. Banana is one of the potential source of prebiotic, while lactobacillus acidophilus is one of probiotics that has superior ability in producing lactic acid to suppress pathogen bacteria. Objective: To determine organoleptic, cholesterol levels and total colony of Lactobacillus acidophilus on piskebal (pisang kepok bakteri asam laktat) synbiotic drinks. Method: This study used a Completely Randomized Design consisting 4 treatments on variations in kepok banana flour (0%, 4%, 8%, 12%). Organoleptic assesment used a hedonic tests conducted on 25 panelists, analysis of cholesterol levels carried out using gas chromatography method and total bacterial colony test used TPC method (cfu/ml). Results: Organoleptic assessment on color, flavor and taste most favored on 0% variations of kepok banana flour, while color and flavor on 8% variations was not preferred, and taste on 12% variations was undesirable. The same total colony of lactobacillus acidophillus were on 8% and 12% variation. Results of laboratory tests, the cholesterol levels of each treatment had the same results (<0.01%). Statistical tests showed that differences in color, flavor, taste and total colony had p-value 0.000; 0.000, 0.001, and 0.000. Conclusion: There were differences between organoleptik and total colony with piskebal synbiotic drinks on each variation of kepok banana flour, while there were no differences on cholesterol level.

Keywords: Synbiotic, Organoleptic, Total Colony, Cholesterol Levels, BAL

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Results of Some Molecular Biological Studies on Local Strains *Lb.plantarum lab3c, lab 19x, lab58a and lb. casei lab 26a, 53a*

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ABSTRACT

Pure cultures of biological active lactic acid bacteria *3c,19h, 26a, 53a,58a* were obtained from fermented mare's milk(airag) and fermented camel's milk (khoormog) which were fermented by Mongolian traditional method also these bacteria were taken from lamb's gastrointestinal tract (cud). Those cultures (*3c,19x, 26a,53a, 58a*) were isolated by izoamil phenol chloroform. DNA purity and output were very good (*3c*- 377.53 ng / *19x*-3052.87 ng / µl, *26a*-2500 ng / ml, *53a*-475.73 ng / µl, *58a*-364.63 ng / µl). The determination for the types of biological active lactic acid bacteria (*3c,19x,26a,53a,58a*) was determined using 8 primers *(SS1, CA1, SS2, HE1, Lfer3, Lfer4, Lpla2, Lpla3)* by molecular biological method. The study results showed that cultures with *3c, 19x, 58a* were *Lb.plantarum,* and *26a and 53a* cultures were confirmed as *Lb.casei*. Among them, the strain of *53a* is composed of 98-99% of strain *Lactobacillus casei* strains in sequence in the NCBI BLAST search. In *Lb.plantarum* and *Lb.casei* local strains, polyacrilamide gel electrophoresis reaction with dodecyl sulfate sodium was tested to assess the purity of bacteriocin protein and the results showed that the local strain of *Lb.casei* sample contained bacteriocin about 46 kDa. These cultures were inhibited growth of pathological bacteria as *Escherichia coli (E.coli 09, E.coli 026, E.coli 10963, E.coli 10977), Salmonella (Sal 0068, Sal SA27), Staphylococcus (Staph.aures 5695, Staph.aures 5068)* of local strains growth in vitro *19h, 53a, 58a* cultures were inhibited 100% of growth of pathological bacteria in vitro and *3c, 26a* cultures were inhibited 87.5% of growth.

Keywords**:** local strain, DNA, primer, sequence

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Down-Regulation of Gluconeogenic Gene Expression by Indigenous *Lactobacillus rhamnosus*

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ABSTRACT

Intake some lactic acid bacteria (LAB) were able to inhibit the progression of diabetes mellitus (DM) by reducing blood glucose level. This study investigated the ability and mechanism of *Lactobacillus rhamnosus* BSL and *Lactobacillus rhamnosus* R23 on lowering fasting blood glucose (FBG) in diabetic rats induced by streptozotocin (STZ). The rats were divided into four groups (n=6), i.e. (N) non-diabetic control group, (DM) diabetic control group, (DM+BSL) diabetic group-treated by *L. rhamnosus* BSL, (DM+R23) diabetic group-treated by *L. rhamnosus* R.23. The rats were oral administered with *L. rhamnosus* BSL and *L. rhamnosus* R23 by giving 1 mL cell suspension (10^9 CFU/mL) daily for 30 days. The FBG was recorded once in six days. Fecal samples were collected on day 0 and 30 for LAB population. Oral administration of *L. rhamnosus* BSL and *L. rhamnosus* R23 decreased FBG and improved glucose tolerance via down-regulation of glucose-6-phosphatase (G6pc) expression by 0.57 and 0.60 fold change respectively ($P<0.05$). The effectiveness of both strains to reduce FBG was well supported with the increased of fecal LAB population on DM+BSL and DM+R23 group after 30 days. These results demonstrated that *L. rhamnosus* BSL and *L. rhamnosus* R23 could passed through in digestive tract and survive detrimental conditions including low pH (in the stomach) and bile salt (in the gut) thus significantly increased the number of LAB population. These findings suggested that *L. rhamnosus* BSL and *L. rhamnosus* R23 has potential as probiotic foods and become a promising agent to manage DM. Further studies on human are required to clarify this anti-diabetic effect.

Keywords: diabetes mellitus, gluconeogenesis, indigenous probiotics, anti-diabetic

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Yogyakarta, August 29-31, 2019

Anti-Bacterial Peptides Isolated from *Bifidobacterium bifidum* in Young *Ovis Aries*

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ABSTRACT

We have investigated the relationship between structure and anti-bacterial activity of peptides produced by lactic acid bacteria and bifidobacteria. In this study, we isolated anti-bacterial peptides from Mongolian young livestock animals and investigated the anti-bacterial activity. Thirty-six single colonies were totally obtained from the 15 faeces and rumen cud samples of Mongolian young livestock animals collected in three provinces (Tuv, Khovd, Khuvsgul). Five bifidobacteria, *Bifidobacterium bifidum*, *B. catenulatum*, *B. ruminantum*, *B. longum*, and *B. pseudocatenulatum*, and 8 lactic acid bacteria, *Enterococcus alcedinis*, *E. faecalis, E. faecium, E. hirae*, *E. lactis*, *E. mundti*, *Lactobacillus reuteri*, and *L. plantarum* were identified by the 16S rDNA sequencing analysis.

We found that *B.bifidum* (BBLA72) isolated from a lamb rumen was produced acetic acid (29.6 mmol/L) and lactic acid (19.3 mmol/L), and the pH of supernatant decreased from 7.0 to 4.57. However, the contents of organic acids were not so much compaerd with those of other ~~strains~~ bifidobacteria~~,~~. It is noteworty for *B.bifidum* (BBLA72) that the cell-free supernatant at pH 7.0 had potent antibacterial activity, which was measured by the diffusion method against both *E. coli* and *Bacilus subtilis*. After 18 h cultivation, the antibacterial activity ~~is~~ was found to be show the. The isolated BBLA 72 bifidobacterium showed pH stability in the wide range of 1-10, especially between the pH of 5 and 7 had potent antibacterial activity and had 16000 AU/mL against *E.coli*. From these results, we considered that the potent antibacterial activity should be due to peptides produced by BBLA

72 bifidobacterium. The peptides showed two clear bands around 2 kDa and 3 kDa on the SDS PAGE and thermal stability for 30 min at 100 C. In addition, after protease treatment of proteinase K and trypsin, the activity disappeared. We continuously investigate the structural analysis of the peptides, explore the antibacterial peptides from Mongolian livestock, and develop the antibacterial peptides for livestock instead of antibiotics.

Keywords: *Probiotic, anti-bacterial peptides, pathogenic bacteria;*

The Effect Of Mangosteen Extract On Viability Of *Lactobacillus plantarum* Mar8 In Black Cincau Ready To Drink (RTD) Beverage

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ABSTRACT

Black cincau is an herbal drink known as a traditional drink to improve fitness and health. As nutraceutical ingredient, it is widely used as antidiabetic, anticancer, antioxidant, antihypertensive, and diarrhea. Mangosteen extract was added to gain more beneficial effect to black grass jelly drinks. It has potential as antioxidant, antiproliferation, anti-inflammatory, anti-obesity and antimicrobes. Probiotication is one effort to add more value to this functional drink. Probiotics are living microorganisms that have beneficial effect for health when consumed in sufficient quantities. The benefits include immune system, and metabolic stimulation, anti-bacteria and improving digestive tract normal flora. Achieving optimal benefits, probiotics must be consumed in sufficient ammount. The recommended dose is 5 x 106 CFU per day minumum for at least 5 days. To determine the effect of mangosteen extract in black cincau drink on *Lactobacillus plantarum* Mar8, a study on the viability of *L. plantarum* Mar8 in this beverage has been conducted. The method was Total Plate Count (TPC). Data showed that after being added to black grass jelly drink, the number of *L. plantarum* Mar8 has decreased. On the second week, *L. plantarum* Mar8 viability in black cincau drink added by 1%, 2% and 3% mangosteen extract was 86.69%, 47.83 %%, and 65.53% respectively. However, cell number decreasing was not significant statistically. Therefore, *L. plantarum* Mar8 potential as probiotic in black grass jelly drinks, because no significant decreasing in cell number. So that, it met the qualification as a probiotic.

Keywords: black cincau, manggosteen extract, *Lactobacillus plantarum* Mar8, viability.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Effect of SCGOS/LCFOS (9:1) + 2'-FL Combination on Infant Gut Microbiota Composition and Metabolic Activities Using *In Vitro* SHIME®

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ABSTRACT

The gut microbiota in early life plays a crucial role in the development of host physiology, metabolism and nutrient absorption. Prebiotics and human milk oligosaccharides have shown to modulate human gut microbiota composition in early life, and promote growth of beneficial gut bacteria and developing the host immune system. In this experiment, we used an *in vitro* gut simulator - SHIME® to investigate the effect of 2'-fucosyllactose (2'-FL), scGOS/lcFOS (9:1) and the combination of scGOS/lcFOS + 2'-FL on the infant gut microbiota composition and its metabolic activity. Fecal sample of a 3-month old C-section born healthy infant, was used to inoculate the colon compartments of a quad-SHIME® to simulate the infant gut microbiota. The SHIME® units each received a modified SHIME® feed un-supplemented (control) or supplemented with either scGOS/lcFOS (9:1), 2'-FL or scGOS/lcFOS + 2'-FL. Samples from the colons compartments were collected over a 2-week period, and the glycoprofiles, short-chain fatty acids (SCFA) and microbiota composition were analyzed. Glycoprofiles showed that 2'-FL was only metabolized in the presence of scGOS/lcFOS across the colon. 2'-FL and the combination of scGOS/lcFOS + 2'-FL resulted in an increased level of butyrate and decreased the level of iso-butyrate (a branched SCFA resulting from the proteolytic fermentation). The 16S rRNA sequencing data showed that supplementation with scGOS/lcFOS + 2'-FL increased the level of *Bifidobacterium, Veillonella* and reduced Proteobacteria, specifically on genus *Klebsiella*. These data suggest that the combination of scGOS/lcFOS + 2'-FL could promote gut maturation and development in early life.

Keywords: 2'-fucosyllactose, scGOS/lcFOS, gut microbiota

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Linamarase-Producing Lactic Acid Bacteria for Cassava (*Manihot esculenta* Crantz) Sourdough Fermentation

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a tropical rootcrop considered as staple food to millions of people worldwide. However, one of the drawbacks of this rootcrop is the presence of cyanogenic compound that can be degraded by lactic acid fermentation. Moreover, linamarase is a hydrolytic enzyme for cyanide degradation and can be produced by lactic acid bacteria (LAB). Thus, this project aimed to screen LAB for their ability to produce linamarase which can then be utilized for cassava sourdough fermentation. A total of 166 LAB from existing collection of cultures and from cassava sourdough were inoculated in a screening medium to determine their ability to degrade cyanide. The resistance of LAB isolates against cyanide was measured using UV spectrophotometer at 600 nm. Isolates with high OD reading were subjected to linamarase assay using p-nitrophenyl-β-D-glucopyranoside (PNPG) as artificial analogue of linamarin. Two isolates from papaya flower (P4 and P15) and one isolate from cassava sourdough (SLI 5) showed high linamarase activities with 50.00 ± 1.88 units/ml, 47.00 ± 2.36 units/ml and 48.00 ± 0.95 units/ml, respectively. These isolates were not significantly different with the reference strain *Lb. plantarum* BS which exhibited a high linamarase activity of 51.67 ± 0.47 units/ml. To ensure that the isolates are safe to be used for the fermentation of cassava sourdough, the isolates were identified through DNA sequencing and BLAST Analysis. Isolates from papaya flower were identified as *Enterococcus faecalis* while the isolate from cassava sourdough was identified as *Leuconostoc mesenteroides* with 100% identity and 99.86%, respectively.

Keywords: *Cassava, Lactic Acid Bacteria, Linamarase*

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Yogyakarta, August 29-31, 2019

Lactic Acid Bacteria from Langsat Fruit In Manado

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ABSTRACT

Lactic acid bacteria have a big contribution in the world of food espesially in producing functional food. Fruits are a natural habitat of lactic acid bacteria because they contain various chemical compositions that are needed as a substrate for Lactic acid bacteria. Lactic acid bacteria can be isolated from various fruits, one of which is Langsat fruit. Langsat fruit is one of the originating fruit from Manado, North Sulawesi, Indonesia. This research aims to obtain lactid acid bacteria from Langsat fruit taken from 4 Districts that is Minahasa District, North Minahasa District, Southeast Minahasa District and South Minahasa District.

50 Acid-producing bacterial isolates were obtained from mature Langsat fruits from 4 districts. 11 acid-producing bacterial isolates were obtained from Southeast Minahasa district, 12 acid-producing bacterial isolates were obtained from the southern district of Minahasa, 12 acid-producing bacterial isolates were obtained from the Minahasa district, and 15 acid-producing bacterial isolates were obtained from North Minahasa district. Media MRS-Agar plus CaCO3 1% is used for the growth of lactic acid bacteria. 42 isolates were conformed as lactic acid bacteria based on gram staining test, catalase, motility, spore formation and gas production from glucose. The characteristics of isolates belonging to lactic acid bacteria in this study were gram-positive, rod shape, negative catalase, negative motility, no spores and homofermentative / heterofermentative. Based on bacterial morphology, the 42 isolates of BAL are rod-shaped so that they are classified into the genus *Lactobacillus.*

Keywords: Langsat, Lactic Acid Bacteria, *Lactobacillus,* Fruit

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Yogyakarta, August 29-31, 2019

Prebiotic Effect of 2 g of Lactulose: A Randomized, Double-blind, Placebo-controlled Crossover Study

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ABSTRACT

[Background]
Lactulose is an indigestible disaccharide produced from lactose that has been used for a long time as a prebiotic material (Bifidus growth factor) in various foods such as infant formula. Although lactulose is used as a laxative, no report has found a quantitative defecation promoting effect compared with a placebo at lower doses. Here, we conducted a randomized, double-blind, placebo-controlled crossover study with 2 g lactulose and placebo to examine prebiotic effects, mainly on the defecation promoting effect.
[Methods]
Sixty healthy Japanese women were randomly assigned to 2-g lactulose or placebo groups. They took test foods for 2 weeks separated by a 3-week washout period. The primary outcome was defecation frequency and the secondary outcomes were the number of defecation days, fecal consistency, fecal volume, and the number and percentage of *Bifidobacterium* in feces. The number and percentage of *Bifidobacterium* in feces were examined using quantitative PCR and a next-generation sequencer, respectively. Straining at defecation for each defecation and flatulence frequency were also investigated.
[Results]
Eight participants were excluded as they did not satisfy the conditions; therefore, data from 52 participants were analyzed. The defecation

frequency (times/week) was significantly higher during lactulose treatment than placebo treatment. The number of defecation days, fecal volume, fecal consistency, and the number and percentage of *Bifidobacterium* in feces were also significantly higher with lactulose than the placebo. Straining at defecation during lactulose treatment was significantly lower than during placebo treatment. No significant difference was observed between lactulose and placebo regarding flatulence. Severe adverse effects did not occur. Thus, oral ingestion of 2 g/day lactulose had a prebiotic effect that increased the number and percentage of *Bifidobacterium* in feces, softened the feces, and increased defecation frequency without increasing flatulence.

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Hemolysis Activity of *Lactobacillus* Local Strains, The Probiotics Candidates

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ABSTRACT

Lactobacillus is part of the normal human gastrointestinal and vaginal flora, it can also be occasionally as human pathogens. Since hemolysis is a common virulence factor among pathogens, the first safety parameter evaluated in vitro was bacterial hemolytic activity. Hemolytic activity of bacteria can be tested by blood agar medium. This study aimed to evaluate the optimal hemolysis activity of *Lactobacillus* local strains on several types of solid media. Nine strains of *Lactobacillus* that had been isolated from mare milk and healthy infant feces (F213, FBB 81, FBB 60, SKG 44, SKG 49, SKG 34, SMM 58, SMM 37.2, and SMM 49) were used in this study. Hemolysis activity was investigated by using cultures streak plate method on blood agar medium, and to perform their microscopic morphology, Gram stain method was used. In this study, the hemolysis was tested by growth of the strains on variety agar base, *i.e.* Blood Agar, Tryptic Soy Agar (TSA), Man, Rogosa, and Sharpe (MRS) agar supplemented with 5% goat blood and incubated for 48 hours at 37°C under anaerobic condition. The results showed that none of nine lactobacillus isolates tested caused beta hemolysis, unless weak hemolytic activity (alpha hemolysis), suggesting that the strains has no hemolytic activity. Both of Blood Agar and TSA base showed the same result while MRS blood agar did not describe hemolysis properly. Their Gram stain morphology showed Gram-positive, rods. The catalase was negative. From this study indicated that Tryptone Soy Agar was the suitable agar base for

blood agar plates used for evaluating hemolysis among *Lactobacillus* strains, which showed hemolysis properly than the other agar base, probably because it is supported by dextrose present in Tryptic Soy Agar medium provides a rich source of carbohydrate that utilized by *Lactobacillus* fermentation and produce lactic acid as a major end product. This finding can be use as consideration when performing hemolytic activity assessment among probiotics candidates.

Keywords: *Lactobacillus*, blood agar, hemolysis, safety assessment

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Yogyakarta, August 29-31, 2019

Isolation, Screening and Identification of Γ-Aminobutyric Acid Producing Lactic Acid Bacteria From Indonesian Fermented Foods

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ABSTRACT

γ-aminobutyric acid (GABA) is a four-carbon free amino acid and act as inhibitory neurotransmitter in the sympathetic nervous systems. GABA is widely distributed in plants, bacteria and vertebrates. Naturally synthesized of GABA are more preferable rather than chemical synthesis due to corrosive reagents of the reactant compounds. Several species of lactic acid bacteria (LAB) have ability to convert monosodium glutamate (MSG) to GABA. Aim of this study was to isolate, screened and identified LAB from Indonesian fermented foods as GABA-producing bacteria. Twelve fermented foods were considered in this study and GABA-producing bacteria were screened using TLC and pre-staining chromatography. Six isolates (IFK10, IFK11, FN12, FN14, FN15) were showed as promising GABA producer and able to convert MSG to GABA during 24 h at 37°C. Two strains IFK10 and IFK 11 was the most efficient strains for the conversion of 2% MSG to GABA within 48 h, with GABA concentration 2.68 mg/ml and 2.06 mg/ml respectively. Based on phylogenetic tree 16S rDNA sequences, strains IFK10 and IFK 11 were identified as *Lactobacillus plantarum*. The open reading frame (ORF) of glutamate decarboxylase (*gad*) gene was amplified by PCR and it consist of 1410 bp. This study indicate that LAB from Indonesian fermented foods could be of interest for the GABA-enriched foods for the development of functional foods.

Keywords: GABA, Indonesian fermented foods, glutamate decarboxylase, *Lb. plantarum*, lactic acid bacteria

10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

Construction of Expression Vector with A Bile-Responsive Promoter In *Lactobacillus plantarum*

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ABSTRACT

Lactobacilli are considered as a potential cell factory because of its GRAS (generally recognized as a safe) status, health-promoting properties and long history of use in food applications. Their ubiquitous nature enables them to be efficient delivery vectors of heterologous proteins. An effective promoter is one of key factors for the controlled and efficient expression of the desired protein. This study aimed to develop a bile-responsive expression system in lactobacilli. Through transcriptomic and proteomic analyses of *Lactobacillus johnsonii* PF01, four promoters of genes highly expressed by bile addition namely phosphoenolpyruvate-dependent sugar phosphotransferase (mannose-specific), L-lactate dehydrogenase, HPr kinase, and D-alanine-D-alanine ligase were identified. For the analyses of the inducible level and promoter strength, each promoter was amplified by polymerase chain reaction and placed upstream of the β-glucuronidase gene as a reporter. Then, these constructs were cloned into *E. coli-Lactobacillus* shuttle vector pULP2, which was generated by the fusion of pUC19 with the *L. plantarum* plasmid pLP27 beforehand. Finally, the constructed plasmid vectors were introduced into *L. plantarum* for a promoter activity assay. Among the four promoters, the L-lactate dehydrogenase promoter showed the highest activity, and it was maintained stable by the host until 80 generations. A bile-responsive expression vector for *Lactobacillus* spp. can be an effective tool for the bile-inducible expression of bioactive proteins in mammalian intestine.

Keywords: *Lactobacillus plantarum*, Plasmid vector, bile-responsive promoter, L-lactate dehydrogenase, β-glucuronidase.

Development Of Gelato Synbiotic From Soygurt And Lesser Yam (Dioscorea esculenta L.). Review Of Chemical, Microbiology, And Organoleptic Quality

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ABSTRACT

Gelato is a product of frozen dairy food dessert with ingredients of milk, whipped cream, egg yolk, stabilizer, and sugar. Sinbiotic gelato is a product of a combination of probiotics and prebiotics that is useful as one of the functional food products containing bioactive components such as antioxidants, isoflavones, fatty acids, dietary fiber, and oligosaccharides. The ingredients used are soybean extract as the main ingredient in milk substitutes, and fermented soygurt using Lactobacillus plantarum B1765 as a probiotic, and substitute for whipped cream, and gembili as stabilizer, and prebiotics. This study aims to determine the chemical quality (pH), microbiology (Total BAL), and organoleptic (Texture, and Taste) contained in synbiotic gelato with the addition of lesser yam (Dioscorea esculenta L.) of 5 grams, 10 grams, 15 grams, 20 grams, and without the addition of lesser yam as a control. The research design used the post test only control group design. Analyzed pH using a pH meter, Total BAL using a Pour Plate Agar technique and was calculated using the Total Plate Count (TPC), and organoleptic hedonic tests analyzed on 30 panelists. The results showed synbiotic gelato with the addition of lesser yam of 5 grams, 10 grams, 15 grams, 20 grams, and without the addition of lesser yam as a control did not significantly influence (P>0,05) to total BAL, and significantly affected (P<0,05) to pH, and organoleptics (texture and taste).

Keywords: Gelato synbiotic, Soygurt, Lesser yam (Dioscorea esculenta L.), Chemical quality, microbiological quality, organoleptic quality.

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Optimization of Culture Conditions for The Growth of *Bacillus coagulans* TM3

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ABSTRACT

Probiotics are living microorganisms that provide a beneficial health effect to the host when ingested in an appropriate amount. Some of *Bacillus coagulans* strains are known to have probiotic properties. This bacterium produces lactic acid, same to lactic acid bacteria, and also been regarded as GRAS (generally recognized as a safe) status. In addition, its spore forming ability allows it to be highly stable in harsh conditions, such as low pH and bile salts in gastrointestinal tract. Bacillus coagulans TM3 was isolated from rice straw. Factors including incubation temperature, pH level, agitation settings, and growth media components were considered during culture condition optimization for TM3. For optimization of culture media, TM3 was grown in broth with 2g/L NaCl and varying concentrations of yeast extract, glucose, and ammonium sulfate. After recording the growth of TM3 under the different formulations, response surface analysis was done to find the formula for the optimum culture media. *Bacillus coagulans* TM3 showed optimum growth at 50°C and pH7. Results also reveal that higher agitation leads to faster growth for TM3. Response surface analysis calculated the optimum growth media content to be 18g/L yeast extract, 0.2g/L glucose, and 8.2g/L ammonium sulfate. The cell number of TM3 at the optimal conditions reached 9.4 × 10^8 CFU/ml. Further study to enhance of the stability of TM3 strain is under way.

Keywords: *Bacillus coagulans*, Media optimization, Probiotic

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The Identification of Lactic Acid Bacteria Isolated from Thai Fermented Meet Using 16S rDNA Technique

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ABSTRACT

Lactic acid bacteria, SKKL1, SKKP1 and NBK10, were screened and isolated from traditional Thai fermented meats; sausages and Nham. Characterization based on the appearance showed that they were gram-positive, rod-shaped with rounded ends occurring singly in pairs or in short chains. The identification of these isolates was carried out by the molecular technique based on the 16S rDNA gene. The results showed that SKKL1, SKKP1 and NBK10 were *Lactobacillus plantarum* (99% identity). However *L. plantarum* shares its ecological niche closely related to species of *Lactobacillus pentosus* and *Lactobacillus paraplantarum*. Due to the high genotypic and phenotypic similarity among these strains, the use of the 16S rDNA sequence alone could not distinguish these three species. Therefore, the *recA* gene was used as a phylogenetic marker for confirmation at species level. The results revealed that the *recA* gene was successfully amplified by PCR using specific primers (planF and pREV) with the product size of 318 bp, as expected. The result from this study confirmed that isolate SKKL1, SKKP1 and NBK10 belonged to the species *L. plantarum*. Further study will be focused on the applications of these strains since *L. plantarum* is the potential candidate for fermented food products.

Keywords: lactic acid bacteria, 16S rDNA, *recA* gene

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Microbiological, Physico-chemical and Organoleptic Assessment of A Carrot-milk Drink Formulation Using *Lactococcus lactis* SC1, *Lactobacillus plantarum* BS and *Pediococcus acidilactici* 3G3 As Potential Probiotic Starter Cultures

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ABSTRACT

Lactic acid bacteria (LAB) are often used as starter cultures in different types of fermented food and are considered as one of the most important probiotic organisms in fermented dairy products. This study was conducted to evaluate three local isolates of lactic acid bacteria (*Lactococcus lactis* SC1, *Lactobacillus plantarum* BS and *Pediococcus acidilactici* 3G3) as potential starter cultures, singly or in combination, for a carrot-milk drink formulation (20% carrot juice and 80% low fat milk). The LAB isolates were evaluated for their antimicrobial activity and results showed that these isolates can inhibit some strains of *Listeria monocytogenes* and *Listeria innocua* (ZOI of 5-24 mm). Fermented carrot-milk drinks were formulated and then evaluated during four weeks of cold storage at 6±2°C. Based on the results of the antimicrobial activity assay (antagonistic to a number of *Listeria* test strains), sensory evaluation (general acceptability score of 6.0), viability of the LAB as starter culture (maintenance of the viability of an estimated 10^8 cfu/ml over a period of 4 weeks of cold storage), and physico-chemical properties of the product (pH of 4.30-4.02 and lactic acid content of 9.96-22.06 g/L), it was concluded that *Lb. plantarum* BS was the best starter culture for the formulated carrot-milk drink. The product formulated in the study could be a potential probiotic drink and can also be considered as functional food due to the presence of β-carotene and other nutrients from the milk and carrot-juice.

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Synergistic Effect of *Lactobacillus gasseri* and *Cudrania tricuspidata* on Anti-Obesity in High-Fat Diet-Fed Mice

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ABSTRACT

Obesity is a global problem in the world. Especially, high-fat diets have increased overweight and obesity problem. Many studies reported the benefits of probiotics and prebiotics, including reduction of weight gain, health promotion, and immunity. A total of 36 mice (C57BL/N6) of 6 weeks-age were divided into 5 groups which were Normal group (NORM, basal diet), High-Fat Diet group (HFD, basal diet+10% fat), and LG, CT, LG+CT groups fed with high-fat diet with *Lactobacillus gasseri* (LG), *Cudrania tricuspidata* (CT), and mixed (CT+LG), respectively. Initial and final body weight were measured at 0 and 10th week, respectively. Afterwards, the mice were sacrificed for fecal microbiota analysis. Compared to HFD group, the LG+CT group showed the highest reduction (11.9%) in body weight gain ($P < 0.05$). In the phylum level, Bacteroidetes was increased in LG, CT and LG+CT groups compared to HFD group. Moreover, Proteobacteria was decreased upon administration of LG and/or CT. In the genus level, remarkable changes in the abundance of some genera known to be involved with leanness were observed. Interestingly, *Helicobacter* which is known to induce inflammation in the gut was drastically reduced in LG, CT and LG+CT groups. In this study, we elucidated the potential anti-obesity effect of combination of *L. gasseri* and *C. tricuspidata* through *in vitro* mice experiment. Future studies should demonstrate the underlying interaction between host and synbiotics.

Keywords: *Lactobacillus gasseri*, *Cudrania tricuspidata*, Gut microbiota, Next-generation sequencing, Anti-obesity

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Simulator of The Human Intestinal Microbial Ecosystem (SHIME®): Effect of Prebiotic Mix Combinations on Gut Microbiota Composition And Short-Chain Fatty Acids (SCFAs)

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ABSTRACT

Prebiotics are dietary components that are also substrates utilized by microorganisms to confer a health benefit to the host. The aim of this study was to investigate the effect of a specific prebiotic fibre combination and quantity (inulin and long chain fructo-oligosaccharides (lcFOS), ratio 9:1, 1g/100ml) compared to short chain FOS (0.4g/100ml) on the eco-physiology of the gut microbiota in early life using the *in vitro* gut simulator SHIME®. An un-supplemented SHIME setup without prebiotics was included as a control. Faecal samples from a 2-year-old, healthy toddler with no antibiotic usage for 3 months were used to inoculate the colon compartments and simulate the infant's gut microbiota. Samples from the colon vessels were collected throughout a 2-week simulation period, and microbiota composition and short-chain fatty acids (SCFAs) were analysed. Both prebiotic mixes stimulated an increase in SCFAs production, with butyrate being the main contributor. Interestingly, only the specific prebiotic mix with inulin and lcFOS stimulated an increase in acetate and propionate production in the distal colon. These SCFAs have been described to have anti-inflammatory effects, promote colonic health and the host's energy metabolism. An increase of *Eubacterium rectale-Clostridium coccoides* group was observed, which in line with transition of a more "mature" microbiota profiles in healthy toddlers. Thus, our study provides insights that inulin/lcFOS enhanced SCFA production quantitively and qualitatively, and may support healthy gut microbial functionalities.

Keywords: prebiotic mix, gut microbiota, SCFA

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Bifidobacterium breve B-3 Promoted Oxidative Muscle Fiber Composition through Activation of AMPK-PGC1α-Mitochondrial Biogenesis Pathway

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ABSTRACT

Bifidobacterium breve B-3, which originated from the gut of an infant, has shown effects lowering body fat along with an increasing of muscle mass in a previous clinical study. We aimed to investigate the underlying mechanisms of B-3 on muscle functions using animal models. Fifty Crl:CD (SD) rats were divided into four groups for oral administration of live B-3 (B-3L, 1×10^9 cfu/body/day), heat-killed B-3 (B-3HK, equivalent cell number to the B-3L group), leucine (positive control for muscle hypertrophy) or saline as control for 4 weeks. We observed a significant increase of the soleus (a slow oxidative (SO) fiber-dominant muscle) per total body weight in the B-3HK group compared with the control group. B-3HK significantly promoted oxidative muscle fiber composition in gastrocnemius muscles and increased phosphorylated AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma coactivator (PGC)-1□, cytochrome c oxidase (CCO) genes expression in the soleus. AMPK and PGC-1□ are involved in shifting the fiber type distribution to oxidative fibers and promoting mitochondrial biogenesis. In addition, the CCO gene as control enzymes related to the mitochondrial oxidative phosphorylation system (OXPHOS) at the mitochondrial membrane were increased by B-3HK administration, which suggested B-3HK could promote mitochondrial biogenesis through the AMPK signaling pathway. These findings suggest that B-3 supplementation might benefit muscle functions possibly mediated by AMPK pathway activation.

Keywords: *Bifidobacterium*, probiotics, skeletal muscle, AMP-activated protein kinase, heat-killed bacteria

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Improving Perfomance and Health Promoting Effects Of Fermented Milk Using Sweet Potato Powder

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ABSTRACT

Lactobacillus rhamnosus SKG34 is a promising probiotic strain that has been intensively studied especially for development of fermented milk. The strain produced a good flavour and acidity of fermented milk but its texture was quite soft. This study was aimed to improve texture of fermented milk by addition of purple sweet potato powder (PSP) and its health promoting effect. The milk was added with 0%, 2%, 4%, 6%, dan 8% (w/v) of PSP prior to pasteurization process. Pasteurized milk was inoculated with *L. rhamnosus* SKG34 then was fermented for 24 hours at 43°C. The results showed that addition of PSP significantly affected total acidity, pH, antioxidant activity, colour, texture and taste of fermented milk. On the other hand, addition of PSP did not affect population of lactic acid bacteria, arome and overall acceptance. Among those applied PSP concentrations, it was found that addition of 8% PSP produced the best fermented milk with pH 3,73, total acidity 1,25%, antioxidant acivity 90,33 with total LAB population 13,19 log10 CFU/ml. This results demonstrated that PSP is promising used to improve total solid content in production of fermented milk using single starter culture *L. rhamnosus* SKG34.

Keywords: Probiotic, fermend milk, purple sweet potato

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Sensory Evaluation and Microbiological Properties Of Probiotic Yogurt Made With Different Type Of Probiotic Cultures Starter

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ABSTRACT

Incorporation of probiotic bacteria into yogurt is believed could increase the health benefit of yogurt such as reduced the risk of type 2 diabetes mellitus. In this study, the sensory and microbiological characteristic of probiotic yogurt made with 2 types of starter were evaluated. The types of probiotic starter were direct vat set (DVS) cultures and heirloom (HC) probiotic cultures. *Lactobacillus acidophilus* La-5® and *Bifidobacterium animalis* subsp. *lactis* (BB-12®) were used in this study and were obtained from Chr. Hansen Malaysia Sdn. Bhd. Conventional yogurt (CY) was made with *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) and *Streptococcus thermophilus* (St). As much as 2% of starter cultures were added into 10% skimmed milk suspension with 10% of sucrose. Lb:St ratio in conventional yogurt was 1:1, whereas Lb:St:La-5:Bb12 ratio was 1:1:2:2. The mixture was incubated at 38 °C for 6-8 hours. Total lactic acid bacteria and bifidobacteria were examined at 0, 1st, 2nd, 3rd, 4th week. The sensory properties of yogurt were evaluated at the 0 and 4th week. After 4 weeks of storage at 4 °C, the total lactic acid bacteria were slightly decreased ranging from 0.26 log CFU/ml (DVS yogurt) to 0.79 log CFU/ml (conventional yogurt). Total bifidobacteria was slightly decrease from 0.40 to 0.58 log CFU/ml. Result from Triangle test revealed that DVS yogurt has similar sensory properties with CY yogurt whereas HC yogurt has less thickness. In general, the sensory properties of probiotic yogurt (colour, thickness, smell,

taste, texture, and overall) were slightly decreased after 4 weeks of storage at 4 ^{o}C. In conclusion, incorporation of probiotic bacteria in yogurt with DVS starter culture give the better microbiological and sensory properties compare to HC starter culture.

Keywords: probiotic yogurt, Lactobacillus acidophilus La-5®, Bifidobacterium animalis subsp. lactis BB-12®, direct vat set starter, heirloom starter

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Effect of Consumption Indigenous Probiotic Powder of *Lactobacillus plantarum* Dad-13 In Maintaining Health in Obesse Suffer in Yogyakarta: Preliminary Study

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ABSTRACT

World Health Organization (WHO) has defined obesity as abnormal or excessive fat accumulation that constitutes a health risk. Prevalence of obesity in Indonesia increased from 2003 to 2018 from 10,5% to 21,8%. Gut microbiota is reported to affect body weight, especially for individuals with obesity. Probiotic is a good bacteria that can maintain gut microbiota balance in the colon. The aim of this research was to know the effect of consumption of indigenous probiotic powder for gut health of subjects with obesity. This research was conducted at Yogyakarta with *randomized-double blind placebo control trial* design for 100 days which were 10 days for the baseline period and 90 days for the ingestion period. Amount 60 obesity subjects ages 44 years old were divided into two groups (the probiotic group who consumed *L. plantarum* Dad-13 $2x10^9$ CFU/sachet and placebo group who consumed skim powder. This research showed that the consumption of indigenous probiotic powder containing *L. plantarum* Dad-13 for 90 days caused significant effect for weight loss and BMI ($p<0,05$) for the probiotic group from baseline to ingestion period respectively 84,54±17,64kg to 83,14±14,71kg and 33,10±6,15 to 32,57±5,01. There was no significant effect for weight loss and BMI ($p>0,05$) for placebo group from baseline to

ingestion period respectively 79,37±11,76kg to 78,80±11,77kg and 31,80±3,71 to 31,56±3,67. (p>0,05) for lipid profile. There was no significant effect for lipid profile and fecal characteristic. The conclusion from this research was the consumption of indigenous probiotic powder containing *L. plantarum* Dad-13 for 33 days tend to decrease body weight for individuals with obesity.

Keywords: obesity, BMI, lipid profile, fecal characteristic, *L. plantarum* Dad-13

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Yogyakarta, August 29-31, 2019

Effect of Regular Consumption of The Probiotic *Lactobacillus paracasei* Strain ET-22 on The Intestinal Microbiota of Mice

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ABSTRACT

In vitro and in vivo studies were carried out to characterize the potential benefits of regular consumption of the putative probiotic *Lactobacillus paracasei* strain ET-22. Acid and bile salt tolerance assays confirmed that ET-22 is well suited to transiting the gastrointestinal tract, with viable ET-22 numbers remaining above 1×10^5 CFU/g after 7 hours exposure. In addition, *Lactobacillus paracasei* ET-22 was demonstrated to have excellent adhesion properties as determined using a Caco-2 cell assay with ET-22 bacterial numbers averaging 120 per Caco-2 cell. To investigate the direct impact ET-22 can have on the intestinal microbiota, 36 healthy SPF BALB/c mice were randomly divided into a blank group, control group and test group (consuming ET-22). The gavage intervention time was 14 days and the ET-22 group received a dose of 0.2 mL/10g body weight (1.3×10^7 CFU/mL). Following the intervention the microbiota was evaluated using a 16S sequencing approach and for the *Lactobacillus paracasei* ET-22 test group, the content of *Bifidobacterium, Lactobacillus* and *Enterococcus* in the intestinal microbiota were determined to have increased significantly over baseline levels by 1.24 log, 0.77 log and 0.36 log respectively in comparison to the blank group (p<0.05?). In conclusion, *L. paracasei* strain ET-22 appears well suited to application as a probiotic for the beneficial modulation of the intestinal flora having acid and bile resistance, strong adhesion to human intestinal epithelial cells, and beneficially impacts on the intestinal microbiota of mice when consumed regularly.

Keywords: *Lactobacillus paracasei* ET-22, intestinal microbiota, probiotic, adhesion

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Growth of Lactic Acid Bacteria and Acid Production in Jack Bean Milk (*Canavalia ensiformis* (L.) DC.) Added with Sucrose and Skim-Milk

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ABSTRACT

The aims of the research were to investigate the ability of indigenous lactic acid bacteria *Lactobacillus plantarum* Dad 13 and *Streptococcus thermophilus* Dad 11 to grow and produce acid in jack bean milk with addition of various concentration of sucrose and skim-milk. The sensory characteristic of the product was also evaluated. Each of 1% v/v *L. plantarum* Dad 13 and *S. thermophilus* Dad 11 was inoculated into jack bean milk with the addition of sucrose (2%-10%), skim-milk (2-10%) and combination of selected sucrose and skim-milk. Incubations were carried out at 37°C for 24 h. Fermentation of jack bean milk without addition of either sucrose or skim-milk was used as a control. The viable cell count, pH value and titratable acidity were monitored in the initial and end of fermentation. These two starter cultures could grow in jack bean milk. Addition of either 2% skim-milk or 2% sucrose increased significantly the production of acid. Further addition of either skim-milk or sucrose (4-10%) did not significantly increase the acid production. Addition of skim-milk improved the physical characteristic of fermented jack bean milk with very sour flavor meanwhile addition of sucrose produced better sweet-sour flavor. Addition of 8% sucrose and 4% skim-milk in jack bean milk produced viscous texture with sweet-sour flavor after 24 h fermentation at 37°C and increased the number of cells to 1.53-1.90 log cycles, with titratable acidity of 0.82-1.37% and pH 3.98-4.32.

Keywords: jack bean milk fermentation, lactic acid bacteria, sucrose, skim-milk

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Aplication *Pediococcus sp* (*Pediococcus halophillus* and *Pediococcus acidilactici*) on Lactic Acid Bacteria of Jambal Roti (Fermented Fish) with NaCl Variation

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ABSTRACT

Jambal roti is a fermented product from manyung fish, which is quite famous in Java. The term jambal roti refers to the salting and drying of fish. Manyung fish which are easily damaged so that they need to be preserved by salting. Traditional production uses 30% salt, so the product is too salty.Decreased use of salt, allows the development of pathogenic bacteria. This study examined the effect of NaCl concentrations (20, 25 and 30%) on total lactic acid bacteria, total bacteria and total *Enterobacteriace* in the making of jambal of fish (*Arius thalassinus*). The results showed that at 20%, 25% and 30% salt concentration, the total lactic acid bacteria in de Mann Rogosa and Sharpe medium had an increase of 2-5 log cycles in all treatments except in *Pediococcus acidilactici* treatment with 25% and 30% NaCl decreasing 2-3 log cycle. total bacteria in Plate Count Agar medium tends to be stable and rises to 3 log cycles in all treatments. Total *enterobacteriaceae* in Violet Red Bile Agar medium tend to be stable and some experience decreases to 2 log cycles. The data shows that *Pediococcus* sp is able to grow up to 30% salinity and suppressed the growth of enterobacteriaceae. Enterobacteriace growth can be suppressed by *Pediococcus* sp when NaCl levels are lowered during manufacture.

Keywords: jambal-roti, *Lactic acid bacteria, Pediococcus sp*

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Bioprocessing Strategies to Enhance and Develop the Functionality and Added Value on Generic Fermented Rice Nutrient

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ABSTRACT

White glutinous rice (WGR) and black glutinous rice (BGR) amongst cereals, has one of the highest potentials for the development of functional foods and bioingredients. It is commonly consumed in its polished state, but the whole grain can be modified by microbial fermentation to improve their nutritional and functional value. The work will focus on the utilization of filamentous fungus, producing amylolytic enzymes (*Amylomyces rouxii*). This fungus able to grow on various solid foods and starch base-products. During the solid state fermentation (SSF) process, amylolytic enzymes also activated to break down starch and release more sugars and oligosaccharides, especially isomalto-oligosacharides which are potentially prebiotic. The generic fermented rice nutrient (GFRN) from fungal SSF cultures will be subsequently used to support the growth of lactic acid bacteria (LAB) to produce highly functional foods with multiple health promoting properties.

Keywords: Solid state fermentation (SSF), white glutinous rice (WGR), black glutinous rice (BGR), *Amylomyces rouxii*, generic fermented rice nutrient (GFRN), lactic acid bacteria (LAB)

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Effect of Consumption Indigenous Probiotic Powder of *Lactobacillus plantarum* Dad-13 of Students SMPN1 Pangururan on The Population of Gut Microbiota

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ABSTRACT

This study aims to determine the effect of probiotic powder *Lactobacillus plantarum* Dad-13 on the number of *Bifidobacterium*, *Lactobacillus plantarum*, *Enterobacteriaceae*, Short Chain Fatty Acid (SCFA) and fecal characteristic of healthy adolescent. This research was conducted at SMPN 1 Pangururan, Samosir with *randomized double-blind placebo-controlled trial*. 54 healthy adolescents aged 13 to 14 were divided into two who consuming a *powder* product containing skim (placebo group) and probiotic *powder Lactobacillus plantarum* Dad-13 with $1.18x10^9$ CFU/sachet (probiotic group). The results showed that for 33 days of intervention, the number of *Bifidobacterium, L. plantarum*, and *Enterobacteriaceae* did not affected significantly ($p> 0.05$) in probiotic group. The amount of SCFA and fecal characteristics in the placebo dan probiotic group before and after taking the products did not differ significantly ($p> 0.05$). But more subjects from the probiotic group had increased *Bifidobacterium*, *Lactobacillus plantarum*, and decreased *Enterobacteriaceae*. The subjects of the probiotic group experienced more increases in SCFA than the subjects in the placebo group. The conclusion is the consumption of probiotic powder *L. plantarum* Dad-13 has not been able to give a significant influence on changes in the number of gut microbiota, SCFA, and fecal characteristics of healthy adolescents.

Keywords: *Lactobacillus plantarum* Dad-13, *Bifidobacterium; Lactobacillus plantarum; Enterobacteriaceae;* Short Chain Fatty Acid

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Isolation and Characterization of Lactic Acid Bacteria from Legume Soaking Water of Tempe Productions

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ABSTRACT

The aims of this study were to isolate lactic acid bacteria from legume soaking water and to examine their ability to grow and produce acid in jack bean milk. Lactic acid bacteria were isolated from legume soaking water in five tempe productions in Special Province of Yogyakarta, Indonesia. Identification of LAB was carried out based on morphological, physiological, and biochemical characteristics. Twenty-nine LAB were obtained and twelve isolates considered to be homo-fermentative types. Four of these homofermentative isolates which be able to grow at pH 4.4 were further examined their ability to grow and produce acid in jack bean milk. Biochemical identification using API 50 CH and 50 CHL identified them as *Lactobacillus* sp. KKNB1, *L. plantarum* WGK3, *L. plantarum* WGK4, and *L. paracasei* WGK5. All those isolates were able to ferment jack bean milk which was marked by an increase of the number of cells (1.07-1.76 log cycle) and decrease of pH value in jack bean milk after fermentation at 37°C for 24 h. It means that they could utilize carbon sources and other nutrients in jack bean milk for their growth and metabolic activities. Further study should be done to evaluate the possibility of these isolates for starter cultures in fermentation of jack bean milk.

Keywords: legume soaking water, lactic acid bacteria, isolation, jack bean milk fermentation

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The Effect of Prebiotic Ginger Yogurt on Fasting Blood Glucose, HbA1c, Insulin, Blood Pressure and Malondialdehyde in Prediabetes Women

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ABSTRACT

Increased blood glucose levels in prediabetes can increase the oxidative stress characterized by increased levels of malondialdehyde and blood pressure. Prediabetes can also cause insulin resistance and increased HbA1c. Prebiotic ginger yogurt containing inulin can reduce insulin resistance and fasting blood glucose (FBG) through increasing short chain fatty acids (SCFA), carrageenan as a source of fiber can inhibit glucose absorption, ginger contains flavonoids which have antioxidant activity that can reduce malondialdehyde and blood pressure. This study aims to analyze the effect of prebiotic ginger yogurt on FBG, HbA1c, blood pressure and malondialdehyde levels in prediabetes women. This study was a quasi-experimental study with control group pretest-posttest design. The subjects were 28 employees women aged 20-60 years old who were selected using consecutive sampling method according to the inclusion criterias, then divided into 2 groups. The treatment group was given 200 ml sinbiotic ginger yogurt for 30 days and nutrition education, while control group was only given nutrition education. Measurement of insulin and malondialdehyde levels was carried out using ELISA method, while HbA1c used immunoturbidimetric assay method. Subjects food intake during intervention was obtained by 24 hour food recall 3 times per week. There was a significant decrease in GDP, HbA1c and blood pressure levels in both groups before and after the intervention ($p < 0.05$). However, there was no difference in decreasing GDP, HbA1c and blood pressure between the treatment group and the control group ($p > 0.05$). Decreased insulin and malondialdehyde levels were not significant in both groups ($p > 0.05$). The

decrease in insulin and malondialdehyde levels did not differ significantly between the treatment group and the control group ($p > 0.05$). The administration of 200 ml of synbiotic ginger yogurt for 30 days had no significant effect in reducing FBG, HbA1c, insulin, blood pressure, and malondialdehyde levels in prediabetes women.

Keywords: prebiotic ginger yogurt, FBG, HbA1c, blood pressure, MDA, prediabetes women

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Adhesion Activity and Antimicrobial Resistance Profile oF *Weissela confusa* Probiotics Strain

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ABSTRACT

Development of Lactic Acid Bacteria (LAB) as probiotics has been widely investigated nowadays. The effects exerted by the LAB are known to be *strain specific*. Therefore, each strain that successfully have been isolated must be further characterized both for their safety aspects and the functional activities. To stimulate the effect on gastrointestinal mucosa, LAB must be successfully adhere on intestinal cells, and then colonize to show their effect. Furthermore, the profiles of strains' antimicrobial resistance were important to be elaborated. Our group has successfully isolated LAB that shows probiotics properties. The adhesion capacity and antimicrobial resistance profiles of the strain have not been investigated yet; therefore, aims of this study were to determine the adherence activity and antimicrobial resistance profile of *W. confusa* F213 strain. The strain, *W. confusa* F213 isolated from

healthy infant feces and showed probiotics properties from previous studies, was included in this study. Post-confluence Caco-2 cells monolayer was used as an *in vitro model* as enterocytes-like cells, and after incubated with the strain; the cells were stained with Giemsa. The adhesion activity was determined with certain criteria. Antimicrobial resistance activity was performed using modified disk diffusion method, while detection of resistance gene was conducted using PCR with primer specific. The adhesion assay showed that *W. confusa* F213 strain has strong adhesion to Caco 2 cells, suggesting that the strain may attach to and colonize in intestine to exert it probiotics effect. Antimicrobial resistance pattern showed resistant to Penicillin, Cefoxitin, and Vancomycin. In concordance with the phenotype results, the antimicrobial resistant encoded genes, *VanH* and *bla*, have been found in this strain. Further identification whether the gene can be horizontally transferred to other bacteria is necessary to be investigated.

Keywords: *Weissella confusa, adhesion, colonization, Caco-2 cells, antimicrobial resistant*

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Development of Kefir with Adding of Dragon Fruit Peel Extract as a High Antioxidant Drink

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ABSTRACT

Diarrhea is one of the endemic diseases in Indonesia that can potentially have an outbreak. In 2015 the prevalence of outbreaks of diarrhea was eighteen times spread across eleven provinces and eighteen cities in Indonesia. One alternative drink for diarrhea sufferers is kefir with addition of dragon fruit peel extract. This study aims to determine the characteristics of kefir with addition of dragon fruit peel extract as a high antioxidant fermented drinks for diarrhea sufferers. The research design used Randomized Block Design (RBD). The treatment used ratio cow milk and dragon fruit peel extract. Cow milk from 75-100% and dragon fruit peel extract from 0-25% with 4 replications. The analysis used in kefir were total lactic acid bacteria, pH, total acid number, antioxidant activity and organoleptic. The results showed that the number of total lactic acid was range from $4,4x10^7$ – $22,3x10^{10}$ CFU/ml, antioxidant activity 20,23-39,95%, pH 4,45-4,61, total acid number 0,37-0,46%. Hedonic quality test showed the more extracts were added, the colors tend to be red, the aroma and taste tend to be sour and thick texture. Panelist tend to like kefir with addition of dragon fruit peel extract. The best treatment is 85% kefir milk and 15% dragon fruit peel extract.

Keywords: Diarrhea, BAL, Probiotics, Kefir Milk, Dragon Fruit Peel Extract

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Antioxidant Activity, Fiber, Acidity (Ph) and Population of Lactic Acid Bacteria (Bal) In Green Juice As Functional Beverages

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ABSTRACT

Pak coy (*Brassica rapa L*) is a vegetable group of brassiceceae which still a family with chinese vegetables. Has contains phytochemical substances such as carotenoids, beta carotene, and glucosinolates which are a source of antioxidant. Pak coy can be processed into juice. The purpose of this research is to determine the content of antioxidant activity, fiber, acidity (pH) and population of lactic acid bacteria in each formulation. The ingredients added in making this green juice are yogurt and honey made into 5 Formulation. The analysis of this research was the analysis of antioxidant activity content using DPPH method (2.2,-diphenyl-1-pikrihidrazil), crude fiber contents analysis using gravimetric method, pH analysis using pH meter and analysis of lactic acid bacteria using spread plate method. The result obtained is the highest content of antioxidant activity was found in formulation 5 consist of 30 g yogurt, 15 g honey and 50 g pak coy with 35,198 ± 0,057%. The content of crude fiber ranged from (0,447 ± 0,046) g to (0,507 ± 0,058) g. The highest of acidity (pH) is in formulation 1 which consist of 10 g of yogurt, 15 g of honey and 50 g of pak coy at 5,50 ± 0,040. The highest population content of lactic acid bacteria in formulation 5 consist of 30 g yogurt, 15 g honey and 50 g pak coy at 2,8 x 10^6 cfu/ml. Therefore green juice can be use as functional beverages.

Keywords: pak coy, green juice, antioxidant activity, crude fiber, acidity (pH) and population of lactic acid bacteria.

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Phenotype and Genotypes Antibiotic Resistant Profiles of *Lactobacillus* Local Strains

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ABSTRACT

Probiotics are living microorganisms that help improve the health of the digestive systems of animals or humans. *Lactobacillus* as a probiotics candidate can be isolated from many fermented food or drink, including mare milk. Our research group has succeeded in isolating and characterized *Lactobacillus rhamnosus* from mare milk (SKG strains) that showed probiotics profiles. Safety aspect of these strains is also important before general application for probiotics candidate. One of safety assessments is their antibiotics resistant profile; therefore aims of this study were to determine the phenotype and genotype profiles of antibiotic resistant of SKG strains. Two strains *L. rhamnosus* (SKG 49 and 34) were used in this study. Phenotype profiles were investigated by using modified disk diffusion method, while genomic and plasmid DNA used for genotypic detection for antimicrobial resistance genes with PCR. Phenotype resistance profiles of these strains showed all strains were resistant against Cefoxitin, Vancomycin, Aminoglycoside, Trimethoprim-Sulfametoxazole and Metronidazole. PCR of genes *bla* (for beta lactamase) and *vanH* (one of Vancomycin resistant encoded gene) showed positive results, supporting Beta Lactam and Vancomycin resistant phenotypically. These findings are important for further safety application in developing functional food product. The safety of lactic acid bacteria is very important, therefore routine evaluation needs to be guided by establised criteria, guidelines and regulations.

Keywords: *Probiotic, Lactobacillus rhamnosus, Beta Lactam, Vancomycin, Bla, VanH.*

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Potential of *Lactobacillus plantarum* B1765 in Fermentation of Soybean Extract as Antihyperglycemic AlternatiVE therapy

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ABSTRACT

Short chain fatty acids (SCFA) have a role in stimulating the hormone GLP-1 in improving the function of β-pancreatic cells in patients with diabetes mellitus (DM) type 2, which occurs due to insulin resistance. SCFA can be produced during carbohydrate fermentation such as stakiosa and rafinosa in soybean extract using strains of L. plantarum. This study aims to determine the effect of certain fermentation time on soybean extract on the pH value and total BAL. The research design used was the post test-only control group design with variations in the form of fermentation time. Samples of fermented soybean are made on a laboratory scale using starter culture L. plantarum B1765. All samples were made in duplication where analysis was carried out using a pH meter and total plate count (TPC) method at 0, 12, 24, 36, and 48 fermentation hours, with a storage temperature of 4^{o}C. The results showed that fermentation time had an effect on the pH value and total LAB. The optimal fermentation time on the BAL growth curve occurs at the 24th hour of fermentation as indicated by the amount of BAL of 6.7067 CFU/g and a pH value of 4.2. There needs to be further testing related to the levels of SCFA and lactic acid produced during soybean fermentation at optimal fermentation time in treating patients with DM type 2.

Keywords: *L. plantarum* B1765, Soybean extract, SCFA, DM type 2

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Isolation and Characterization of Lactic Acid Bacteria from RIAU TRADITIONAL TEMPOYAK

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ABSTRACT

Tempoyak is a fermented food made from durian fruit and has a sour taste produced by lactic acid bacteria (LAB). In this study, tempoyak samples were collected from 4 traditional markets in Riau namely Arengka, Panam, Dupa, and Bangkinang. Isolation was carried out by growing the microbes on MRS specific media with the spread plate method. Culture was incubated for 48 hours at 45^{o}C and then colony growth was observed, continued with gram staining of bacteria. The total population of LAB was calculated using the Total Plate Count method. Whilst, characteristics were undertaken by catalase and glucose test. There were 50 isolates characterized as gram-positive bacteria with basil form. Macroscopic morphological observations showed that all isolates were circular with convex elevation. The average total population of lactic acid bacteria obtained ranged from 10x10^{1}-12,65 x 10^{4} CFU/ml. The results also showed that all isolates were negative for catalase, whereas there were 48 glucose-positive isolates and 2 glucose-negative isolates. Based on the results of morphological identification and simple biochemical tests, 45 LAB isolates were identified as *Lactobacillus* because of their forms and ability to live at 45^{o}C, negative catalase, glucose positive, and were gram-positive bacteria.

Keywords: durian, fermentation, lactic acid bacteria, tempoyak

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Viability of *Lactobacillus plantarum* in cocoa powder and dark chocolate compound

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ABSTRACT

Lactobacillus plantarum has been known to have probiotic properties. The trend of healthy lifestyle encourages the development of probiotic functional food in the market. In order to be considered probiotic products, the amount of probiotic in food matrices must be at least 6 log CFU/g at the end of storage. To bring more added value to the product, the viability of probiotic bacteria in food matrices must be enhanced with the use of protective ingredients and the application of microencapsulation. In this study, cocoa powder and dark chocolate compound are chosen as ingredients to lengthen the viability of added *L. plantarum*. Treatment groups were varied for different form of *L.plantarum* cells (free cells and encapsulated cells) and the addition of different ingredients (cocoa powder and chocolate compound). For all treatment groups, the viability of *L.plantarum,* both free cells and encapsulated, was able to be maintained above 6 log CFU/g after a week of observation. Statistical evaluation of treatment groups showed no significant difference when compared to each other at 95% confidence level. It can be concluded that the addition of cocoa powder and dark chocolate compound is potential to be further explored as protective ingredients for probiotic.

Keywords: *Lactobacillus plantarum*, viability, encapsulation, chocolate compound, cocoa powder.

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Assessment of Microorganisms Isolated From Commercially-Available Probiotic Products Suitable for Children Being Marketed In The Philippines

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ABSTRACT

A high mortality rate for infants and children in the Philippines has been noted due to persistent and acute watery diarrhea. Doctors are now looking into the use of probiotic products as diet supplement and therapeutic drug to aid in the treatment of acute watery diarrhea. However, for a probiotic to be effective, it must be viable, in adequate amount, must survive passage in gastrointestinal tract, and has antimicrobial properties. This study evaluated five probiotic products for children available in the Philippines in terms of the microorganisms' survival in gut-simulated conditions and their antimicrobial activities. After culture isolation and molecular identification, microorganisms were subjected to acid tolerance test, bile tolerance test, and agar diffusion method. Some microorganisms recovered, such as *Lactobacillus reuteri and L. paracasei*, were found to survive low pH and can tolerate the presence of bile. Lysates of *Lactobacillus* isolates were found to exert antagonistic activity against common gut pathogens like *Escherichia coli* O157:H7, *Salmonella enterica, Klebsiella pneumoniae, and Proteus vulgaris*. This study is the first to assess microorganisms in commercially-available probiotic products intended for children in the Philippines for their survival in acid and bile stress as well as antimicrobial properties *in vitro.* It can be used as a baseline for establishing stricter guidelines when assessing and approving commercial probiotics in the Philippine market.

Keywords: acid tolerance, antimicrobial properties, bile tolerance, children, probiotics

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The Effect of Consumption Indigenous Probiotic Powder Containing *Lactobacillus plantarum* DAD-13 Of students SMPN 1 Pangururan, Samosir on Weight, Body Mass Index and The Population of Gut MicroBIOTA *Prevotella*, *Bacteroides fragilis* And *Clostridium coccoides*

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ABSTRACT

The human intestine is a very complex ecosystem that inhibited by beneficial microbiota for host health. Some factor that affects microbiota diversity in intestines, such as age and dietary habit. Samosir is one of the regions in Indonesia that have different dietary habits. Probiotics are microorganisms with health benefits, especially in the human intestine. The purpose of this research is to investigate the consumption effect of indigenous probiotic powder, L. plantarum Dad-13, for microbial diversity of Prevotella, B. fragilis, and C. coccoides. The study was conducted at SMPN 1 Pangururan, Samosir for 43 days (33 days intervention). The study was conducted in a double-blind placebo-controlled trial. The treatment group consumed 1 gram of probiotic powder with the viability of 1.18x109 Cells/gram, and the placebo group consumed 1 gram of skim milk. Stool sampling and data on height and weight were collected on the 10th day and 43rd day. The analysis of microbiota using Real-Time PCR (qPCR). From the results obtained height from both groups significantly increased. The body weight significantly increases in probiotic group from 44.35 kg ± 4.61 to 45.20 kg ± 4.78. The BMI values of the subjects from probiotic groups significantly increase. Microbiota analysis showed insignificant results with the consumption of indigenous powdered L. plantarum Dad-13 probiotics for 33 days.

Keywords: Probiotics, Microbiota, Adolescents, Body Mass Index

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Antioxidant And Prebiotics Properties of Single Garlic (*Allium sativum*) Pickle

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ABSTRACT

A single garlic is type of garlic that has a single clove, it is also known as single clove garlic, monobulb garlic or single bulb garlic. Traditionally, single garlic was used as medicinal based on its properties as antimicrobial, antiprotozoal, antimutagenic, antiplatelet, antihyperlipidemic and antioxidant. The alline substance was presumably as antioxidant, but it was changed easily to unstable allicine caused by allinase enzyme activity which increase when garlic was chopped, crushed or processed. The change of alline to allicin will reduced the antioxidant activity of single garlic. A treatment that reduce the allinase activity will retain the alline substance andthe antioxidant capasity could be maintained. Fermentation process will give a low pH as the result of carbohydrate metabolism in a fermentation medium. The aims of this research were to evaluate the growth of *Lactobacillus plantarum* B1765 as a starter culture in single garlic, pH andthe effect of fermentation time process (3, 6 and 9 days)to antioxidant activity on single garlic pickle. The growth of *L.plantarum* B1765 were measured as Total Lactic Acid Bacteria (LAB) by Total Plate Count (TPC) using MRS medium andantioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging methods, expressed as inhibition activity (%). Fermentation process along 9 days showed that *L.plantarum* B1765 growth on single garlic pikles for 1 log cycles from 10^6 CFU/mL to 10^7 CFU/mL, pH reduces from 5.6 to 4.4, closely related to increasing of TAT from 0.16 % to 0.28%. Fermentation process also showed an increasing of the inhibition activity along the fermentation process from 10,67% to 11.17% and showed a higher inhibition than control (3.88%- 7.39%).Single pickle garlic is potentially to be developed as prebiotics and natural antioxidant health food.

Keywords: pickle, single garlic, alliin, antioxidant, prebiotic

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Microencapsulation of Indigenous Probiotic *Lactobacillus plantarum* DAD 13: Strain Dependent Effect and Its Antibacterial Property

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ABSTRACT

Probiotic's viability is substantial for its health benefit, and microencapsulation is a promising method to preserve its viability. Spray and freeze-drying are the common methods, however, its effect on the stability of probiotic is strain-dependent and gives different characteristics. Thus, in this research, *Lactobacillus plantarum* DAD 13 microencapsulation was performed with 10% (w/v) skim milk as wall material by using both methods. Strain dependent effect was observed by comparing the viability with *L. plantarum* MUT 7 after the process and sublethal injury analysis. The stability of probiotic was observed during 8 weeks of storage at room temperature and 4°C. The antibacterial property was performed to evaluate whether the process affects the probiotic's properties. The results show that there was no significant difference in terms of microcapsule characteristics. Hence, the microstructure of spray-dried probiotic was spherical shaped, while freeze-dried probiotic was irregular. However, the viable cell of freeze-dried probiotic after the process was significantly higher with less log redaction (±1 log cycle) compare to spray-dried probiotic (±2 log cycle). Sublethal injury analysis shows that, the survival of freeze-dried probiotic was higher (±99%) compare to spray dried probiotic (±96%). In addition, the inactivation rate of freeze-dried probiotic was smaller than spray dried probiotic microcapsule at analysed temperatures. From both strains, we found that there was no strain-dependent effect due to the different method. Thus, we deduced to choose freeze-drying for microencapsulation *L. plantarum* DAD 13. Last, freeze-dried *L. plantarum* DAD 13 still had the antibacterial property.

Keywords: probiotic, *L. plantarum* DAD 13, microencapsulation, spray drying, freeze-drying, strain-dependent

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Evaluation Of The Probiotic Properties Of *Lactobacillus Paracasei* And *L. Curvatus* Isolated From Bovine Colostrum And Encapsulated In Dry Preparations.

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ABSTRACT

Lactobacillus is one of the genera of probiotics that are widely used to maintain health and prevent disease and commonly encapsulated in dry preparations. In this study will be evaluated the probiotic properties of *Lactobacillus Paracasei* and *L. Curvatus* encapsulated in carriers only in alginate, skim alginate, and tapioca alginate The study was carried out experimentally using a completely randomized factorial pattern. Data were analyzed using Variance Analysis with a 95% confidence level and continued with Duncan's multi-range test. Evaluation of probiotic properties was carried out on cell viability during storage, acidity resistance, resistance to bile salt concentration, and antimicrobial properties of *Escherichia coli* and *Salmonella typhimurium*. The results showed that L. curvatus in the fourth week had high viability of 2.25 x 10^{10} CFU / ml and was resistant to pH 2 in skim alginate, whereas L. paracasei had a cell number of 2.98 x 10^{10} CFU/ ml also resistance to pH 2 in tapioca alginate. The two *Lactobacillus* species were also resistant to the concentration of 0.3% bile salts and 0.5%, which incubated for 6 hours. L. curvatus in the skim alginate formula also has antimicrobial activity against *Escherichia coli* and *Salmonella typhimurium* with each inhibitory diameter of 17.83 mm and 12.00 mm. Encapsulation combination of alginate and skim milk produces high viability for probiotics during processing, storage, and acidic.

Keywords: Encapsulation, *Lactobacillu curvatus, lactobacillus paracasei*, probiotic properties.

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Effect of Consumption of Indigenous Probiotic powder *L. plantarum Dad-13* on Lipid Profile and fecal microbiota of overweight subjects living in Yogyakarta, Indonesia

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ABSTRACT

Recent studies have suggested that gut microbiota play an important role in modulating risk of several chronic diseases, including obesity. Probiotic is considered to have a role in modulating gut microbiota with the impact in maintenance of body weight. *Lactobacillus plantarum Dad-13* is indigenous probiotic isolated from dadih, traditional fermented buffalo milk from West Sumatera, Indonesia. Objective of this study were to evaluate the health benefit of consumption of indigenous probiotic powder *L. plantarum Dad-13* on overweight subjects by evaluating the lipid profile and gut microbiota. Powder of probiotic *L.plantarum Dad-13* with the concentration of $5.5x10^9$ CFU/g were given to 20 volunteers for 90 days (3 months). The result showed that consumption of probiotic powder could decrease The total cholesterol parameters for female overweight subjects by 23.5%. For triglyceride parameters in female subjects overweight decreased by 0.8% while for male subjects overweight experienced an increase of 27.17%. Aligned with cholesterol LDL parameters for female subjects decreased by 24.32% while in male subjects experienced an increase of 17.46%. For HDL cholesterol parameters in both female and male subjects decreased by 26.47% and 40.98% respectively

Keywords: *Indigenous Probiotic Powder, Overweight, Lipid profile, L.plantarum DAD-13, gut microbiota*

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Fermented Food Diversity In South Kalimantan

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ABSTRACT

Fermentation technology is a traditional food preservation technique that utilizes the activity of microorganisms to inhibit the growth of bacterial decomposition and pathogens. Fermented food products have a specific flavor that is not owned by non-fermented products so favored by consumers. Almost all fermented products involve the activity of lactic acid bacteria during the fermentation process. This paper is a scientific study of the diversity of fermented food products in South Kalimantan. Various types of traditional fermented foods made from animal and vegetable are identified in all districts in South Kalimantan. Fermented animal food groups are beef jerky, duck, squid, and eel, peda, wadi, fish wadi eggs, shrimp, shrimp paste, ronto, and salted egg. Vegetable food fermentation group is various kinds of pickled vegetables, pakasam puka, jaruk tigarun, jaruk belimbing wuluh, jaruk tandui, samu kapayang, jaruk tarap, mandai cempedak pulps, kalangkala, nagara bean tempe, glutinous tape, cassava tape and lotus seed tape. Most of the identified products have not been studied in depth, so the nutritional value is not known, the benefits for health, standardization of the way of processing and the quality of the product. The product is found in several districts and is part of the public consumption menu. There is a need for a more in-depth review of all these products as an effort to conserve traditional foods of South Kalimantan and furthermore have the opportunity to serve as a functional food source.

Keywords: fermented foods, diversity, south Kalimantan

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Technological Properties of Antimicrobial-Producing Lactic Acid Bacteria Isolated From Raw Milks and Dairy Products of Sabah (Northern Borneo), Malaysia

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ABSTRACT

This study aims to evaluate the technological properties of the antimicrobial-producing LAB isolated from cow and goat milk, and also various types of cheese. In preliminary identification, the isolated strains were identified by various tests such as Gram staining, catalase test and carbon fermentation. Then, the agar well diffusion assay was performed by utilizing the *L. monocytogenes* ATCC 7644 and *L. monocytogenes* ATCC 13933 as the indicator microorganisms before the biochemical and physiological tests were conducted to assess the beneficial properties of the selected strains. Results show that 5 out of 20 isolated LAB strains were the antimicrobial-producer indicated by the formation of clear inhibition zones. Morphologically, these 5 strains are rod and cocci in shape, catalase negative, able to ferment carbon, and also tolerate to various concentrations of NaCl and wide range of temperatures. Strain CCB1, GB3 and CB3 showed positive proteolytic activity, while CCB1, GB3 and CA1 were able to hydrolyse starch. Other than that, isolates CCB1, CB3 and CA1 have ability to deconjugate bile salt in both aerobic and anaerobic condition. Moreover, CCB1, GB3 and CA4

were susceptible with ampicillin, tetracycline, ceftriaxone, penicillin G and chloramphenicol. However, most of the strains were resistant to norfloxacin, amikacin, colistin sulphate, streptomycin and nalidixic acid. Lastly, in the presence of bile salt and phenol, all of the 5 strains were able to grow and there was no inhibition occur. The newly isolated LAB strains with valuable features might offer an unfolded potency that are beneficial for applications in food industry.

Keywords: Isolation, lactic acid bacteria, food industry, milk products

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Gut Microbiota of Thai Colorectal Cancer Patients

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ABSTRACT

Colorectal cancer (CRC) is the third most prevalent cancer in the world. There is increasing evidence that microbial dysbiosis in the gut contributes to CRC development. However, the relationship between human microbiota and CRC is still unexplored in Thailand. This study aimed to examine gut microbiota in three different groups of subjects. These included 21 participants with no CRC (NO), 6 patients with CRC, and 11 participants with adenomatous polyp (PO). The samples were collected at Bangkok Hospital Phuket. Next-generation sequencing (NGS) was used to analyze and compare human microbiota composition from saliva, feces, and colonic tissue from all subjects. The results showed that species richness of the NO-biopsy samples was highest compared to others. The NMDS plot indicated that salivary microbiota are clearly distinguished from the microbiota of the intestinal lumen. Interestingly, the microbiota of PO and CRC-biopsy samples were separated from other parts of the intestinal lumen. LEfSe analysis between cancerous and non-cancerous microbiota indicated that *Akkermansia muciniphila* might be a biomarker for indicating the cancerous area. Moreover, butyrate-producing bacteria were less abundance in CRC-biopsy samples. Additionally, these samples exhibited higher abundance of Gammaproteobacteria. Taken together, the gut microbiota of CRC Thai patients are unique and distinct from PO and NO participants.

Keywords**:** gut microbiota, colorectal cancer, Thai subjects, biopsy, Thailand

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Antimicrobial Activity of *Lactobacillus plantarum* G-3 Isolated from Dadih Against Pathogenic Bacteria

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ABSTRACT

Dadih is an Indonesian fermented food coming from West Sumatra as it is derived from buffalo milk fermented spontaneously by lactic acid bacteria. It has high nutritional value, contains relatively high protein and fat compared to yogurt from cow milk, and is a rich source of amino acid. As a fermented food, dadih may contain some beneficial bacteria like lactic acid bacteria, which usually have antimicrobial properties such as hydrogen peroxide, organic acid, and a bacteriocin, which is important in the utilization lactic acid bacteria as probiotic agent and food preservative. The purpose of this study was to determine the antagonistic activity of *Lactobacillus plantarum* G-3 isolated from dadih against pathogenic bacteria. The antagonistic assay of 18-24 hours incubation time of *Lactobacillus plantarum* G-3 was conducted in Man Rogosa and Sharpe agar using wall diffusion agar method. The result showed that *Lactobacillus plantarum* G-3 inhibited the growth of pathogens bacteria including *Salmonella sp.*, *Escherichia coli FNCC 0091*, *Bacillus subtilis* FNCC 0059, *Staphylococcus aureus* FNCC 0047, and *Pseudomonas aeruginosa* FNCC 0063 as show by the inhibition zone ranging from 18 milimeters to 25 milimeters. According to a large inhibitory spectrum, our results show that *Lactobacillus plantarum* G-3 can be applied as food preservative and/or as probiotic in the protection of human health.

Keywords: Lactic acid bacteria, pathogenic bacteria, antagonistic activity

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The Effect of Different Methods of Giving Probiotics *Lactobacillus Sp.* on Broilers Performance In Starter Period

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ABSTRACT

The objective of the study was to evaluated the effect of different methods of giving probiotics *Lactobacillus Sp.* on broilers performance in starter period. A total of 200 days old chick were reared until 14 days of age. The completely randomized design with 4 treaments and 5 replications was used in the research. The treatments were T0 = Control without probiotics, T1 = Force feeding method, T2 = Giving *Lactobacillus Sp.* in Feed, T3 = *Giving Lactobacillus* Sp. in drinking Water. The parameters were broilers performance (feed consumption, body weight gain and feed conversion ratio) in starter period with different methods of giving probiotics. The analyzed variance (ANOVA) and tests performed Duncan's Multiple Areas used to obtained the data. The Result showed that there are no significantly on the feed consumption of broilers with different methods of giving probiotics *Lactobacillus Sp.* The feed conversion ratio of broilers in starter period with given probiotics *Lactobacillus* Sp. (T1, T2 dan T3) were lower ($p < 0.05$) but the body weight gain of broilers were higher ($p < 0.05$) compared to control treatment. The conclusion was the Broilers in starter period with given probiotics *Lactobacillus* Sp. in different methods showed the better performance compared to broilers without probiotics.

Keywords: Force feeding, Probiotics, *Lactobacillus* Sp., Broilers, Performance

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The Effects of Consumption of Indigenous Probiotic Powder *containing Lactobacillus plantarum* Dad-13 in Malnourished Children of Belanting Elementary School, East Lombok on The Population of Gut Microbiota *L. plantarum, Bifidobacterium, Enterobacteriaceae* and Short-Chain Fatty acids

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ABSTRACT

Malnutrition is a condition of imbalance between the body's nutritional needs and nutrient intake. In addition to lack of nutrients, pathogenic bacterial infections are also a cause of malnutrition. The purpose of this study was to determine the effects of consumption of probiotic powder *Lactobacillus plantarum* Dad-13 on the population of *Lactobacillus plantarum*, *Bifidobacterium*, *Enterobacteriaceae* and Short-chain fatty acids. The consumption of probiotic powder *L. plantarum* Dad-13 ($1.18x10^9$ CFU / g) was undertaken for 60 days on 40 malnourished elementary school students, who had been divided into placebo and treatment groups. The research design used was *Randomized Double-Blind Placebo-Controlled*. The results showed that the consumption of probiotics *L. plantarum* Dad-13 caused a significant increase in the numbers of *Lactobacillus plantarum* and *Bifidobacterium* ($p<0.05$) but decreased numbers of *Enterobacteriaceae*. The concentrations of the (SCFA) short-chain fatty acids (acetate, propionate and butyrate) in the placebo group did not change significantly ($p> 0.05$), while the probiotics group ($p<0.05$) had a significant increase in SCFA and a significantly reduced pH. The conclusion of this study is that consumption of *Lactobacillus plantarum* Dad-13 can increase good bacteria and Short-chain fatty acids in malnourished children of Belanting elementary school, East Lombok, West Nusa Tenggara.

Keywords: Probiotic powder, malnutrition, *L.plantarum* Dad-13, SCFA.

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Impact of the putative probiotic *Bifidobacterium lactis* strain BL-99 on improving digestion function

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ABSTRACT

The effect on digestion of *Bifidobacterium lactis* strain BL-99, a putative probiotic originally isolated from a healthy Chinese infant, was assessed in a mouse model using a daily supplementation regime. Male Balb/c mice were randomly divided into a control group, model control, and five dosage groups with live *B. lactis* strain BL-99. Both the control and model control groups were administered orally with PBS at a dose of 20mg/(kg.d). For the test groups, strain BL-99 suspensions at concentrations of:,1×10^6, 1×10^7, 1×10^8, 1×10^9, 1×10^{10}cfu/d (human body dose equivalent) were given to mice daily. The impact of the probiotic strain on digestibility was established by the ink propulsion test, and measurements of body weight, food intake, and rat pepsin. After 30 days intervention, the BL-99 very low dose(1×10^6cfu/d),low does(1×10^7cfu/d)and high does (1×10^9cfu/d) groups had significantly higher ink advancement rates compared with the control group ($p < 0.05$) indicating improved digestion rates. The middle dose group ($1x10^8$cfu/d) and high dose group ($1x10^9$ cfu/d) had significantly higher gastric juice secretion than the control model group ($p < 0.05$). The middle does group ($1x10^8$cfu/d) had a significantly higher pepsin excretion than the control group ($p < 0.05$).But no digestion promotion effect has been found at $1x10^{10}$cfu/d. These results indicate that *B. lactis* BL-99 can positively influence digestion and that the dose is important for this effect.

Keywords: *Bifidobacterium lactis*, promote digestion, probiotic, dose response

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Effects of Probiotics *Lactobacillus* on Japanese Quails Production

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ABSTRACT

The aim of this study was to evaluate the probiotic properties of the selected *Lactobacillus* strains to improving the growth performance of Japanese quails. The experiments were divided into two parts. In part I, *Lactobacillus plantarum* SKKL1 and *L. curvatus* FJNP12 were selected to evaluate preliminary probiotic properties. The results showed that all of selected *Lactobacillus* strains could survive in the acid tolerance condition and tolerated to 0.3% bile salt. The selected *Lactobacillus* strains were then tested for an eventual inhibitory action against pathogenic bacteria using agar well diffusion assay. All selected LAB strains produced organic acids to inhibit the growth of the tested pathogenic bacteria. Moreover, all of the selected *Lactobacillus* strains could inhibit the growth of *Sal*. Typhimurium and *E. coli* in co-culture growth experiments. The antibiotic susceptibility test showed that all strains were susceptible to ampicillin, chloramphenicol, erythromycin, gentamycin, penicillin and tetracycline, whereas resistant to kanamycin, norfloxacin, streptomycin and vancomycin. The coexistence test revealed a lack of antagonism against each other of all selected LAB. Thus, these two strains, representing different species, could possibly be combined as a probiotic mixture. In part II, Japanese quails were divided into 5 groups; 1) Basal diet, 2) Bactosac-P (commercial probiotics), 3) *L. plantarum* SKKL1, 4) *L. curvatus* FJNP12, and 5) SKKL1+FJNP12. The tested strains were inoculated into basal diet in the form of single and multi-strain preparations. Results found that in all of the treatments supplemented with probiotics *Lactobacillus*, the numbers of viable cells of probiotics in feed were decreased below log 7 CFU/g feed after 21 d of storage at 4°C.

Keywords: *Lactobacillus*, probiotics, Japanese quails

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Colostrum Yogurt: Antimicrobial Activity And The Effect On Rat Fecal Microbes

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ABSTRACT

Bovine colostrum is the secretion of cows' mammary gland in early lactation until 5-7 days after the birth of the calf. Colostrum has a different composition and sensory characteristics from mature milk, so it is not absorbed by the dairy industry. In this study, colostrum used as raw material in the manufacture of yogurt produced colostrum yogurt. Colostrum yogurt contained antimicrobial compound derived from bioactive compounds in colostrum or metabolites produced by yogurt starter bacteria. This study aims to determine whether the colostrum yogurt products have antimicrobial activity, and the effects of consumption of these products against rats' intestinal microbes. The yogurt starter is used *Lactobacillus bulgaricus* FNCC 0041 and *Streptococcus thermophilus* FNCC 0040, each 6.25% (v/v) of the initial volume of pasteurized colostrum. The homogenous mixed is packaged in polypropylene cup capacity of 100 mL and then incubated at 42°C for 4.5 hours, so the yogurt's pH reaches about 4.6. Yogurt that has been incubated is stored in a refrigerator at a temperature of 4-5°C for 0, 7, and 14 days. Colostrum yogurt has an inhibitory activity against *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus* in vitro testing using the well diffusion and contact method with a small to moderate inhibitory activity. Giving colostrum yogurt in mice for 21 days showed significant decrease (α=0,05) in the number of coliform bacteria in the feces of rats but the amount of lactic acid bacteria are not significantly different between colostrum yogurt treated rats and control rats.

Keywords: colostrum yogurt, antimicrobial activity, faeces microflora

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Isolation and Evaluation of Probiotic Potential of Lactic Acid Bacteria from Oncom

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ABSTRACT

Oncom is one of Indonesian traditional fermented food which made by fermentation of various species of fungi, *Neurospora intermedia* var. *oncomensis* and *Rhizopus oligosporus*. Diversity of microorganism in the consortium has not been thoroughly explored and studied. The aim of the present work was to isolate and examine the potential probiotic properties of lactic acid bacteria (LAB) from oncom, such as production of antibacterial substances, haemolytic activity, low pH tolerance, and bile resistance of LAB isolated. From two red oncom and one black oncom, 11 LAB isolate were isolated and five selected isolates were identified by 16S rDNA sequencing as *Pediococcus pentosaceus* (LR.JBb.1; LR.C.27; LB.Boe.34; LB.Boe.36) and *Lactobacillus plantarum* (LB.Boe.35). In general, all identified LAB were able to inhibit both *Escherichia coli* and *Staphylococcus aureus*. Antibacterial against *Salmonella enterica* was absent in LB.Boe.34 and LB.Boe.36. Alpha-haemolytic activity was observed in LB.Boe.34 and LB.Boe.35. In general, the selected strains showed moderate to tolerate the acidity at pH 2.5 after 3 hours of incubation, but no tolerance to pH 1.0. LAB isolates were able to survive in the presence of 0.3% bile. These findings imply that *Pediococcus pentosaceus* LR.JBb.1 and LR.C.27 appeared as the promising candidates to be used as potential probiotics and might be subjects for *in vivo* studies.

Keywords: Probiotic, acid and bile tolerance, antibacterial, lactic acid bacteria, oncom

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Can *Enterococcus hirae* be a nisin producer?

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ABSTRACT

The purpose of this study was to select a promising bacteriocin producer strain from goat milk for biopreservation. Characterization of the expressed bacteriocin focused on its physiological and biochemical properties, and specificity of the operon encoding production and expression of the antimicrobial peptide. Brazilian goat milk was used as the source for selection of bacteriocin producing lactic acid bacteria. One strain (DF105Mi) in particular showed strong activity against several *Listeria monocytogenes* strains. Based on biochemical and physiological analysis species specific PCR and 16S rRNA sequencing strain DF105Mi was classified as *Enterococcus hirae*. The bacteriocin production and inhibitory spectrum of *Enterococcus hirae* DF105Mi were studied, together with determination of the effect of temperature, pH and chemicals on bacteriocin stability and production, activity and adsorption to target cells as well as to the cell surface of bacteriocin producing strains. Physiological and bio-molecular analyses for targeting different genes and parts of the nisin operon were performed to investigate the hypothesis that the *Enterococcus hirae* strain DF105Mi can produce and express nisin. *Enterococcus hirae* DF105Mi produces a bacteriocin, stable in a wide pH range (2.0 - 12.0), high temperatures (up to 120 °C), in presence of selected chemicals, and presenting adsorption affinity to different test organisms influenced by environmental conditions. Higher bacteriocin production by *Enterococcus hirae* DF105Mi was recorded during stationary growth phase, but only when the strain was grown at 37 °C.

Genetic analysis of strain DF105Mi indicated the presence of the genes encoding the production of the bacteriocin nisin. This result was confirmed by cross-checking the sensitivity of the producing strain with commercial nisin A. The strong anti-*Listeria* activity, bacteriocin adsorption and stability of the produced bacteriocin indicate that *Enterococcus hirae* DF105Mi has a differentiated potential for application in biopreservation of fermented dairy products.

Keywords: bacteriocins; *Enterococcus hirae*; goat milk; biopreservation; nisin operon

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Effects of Probiotic In Pelleted Feed On Growth Performance of Broilers

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ABSTRACT

The objective of this study was to development a potential probiotic product in pelleted feed to improve broiler production performance. In *in vitro* test, we found that three inactivated lactic acid bacteria (LAB) cells, including *Lactobacillus pentosus* LPS16, *L. plantarum* LP28, and *L. acidophilus* LA27 exhibited better or similar adhesion and immunity stimulation abilities compared with live cells. These three LAB were combined with one *Bacillus subtilis* for further study. A total of 280 one-day-old ROSS 308 broilers were randomly distributed into control group (CT group), antibiotic group (Enramycin 10 ppm, AB group), commercial probiotic product group (CM group), and probiotic group (SYN group), with 5 replicate pens per treatment (14 birds/ replicate pen). Antibiotic or probiotic was blended with feed before pelleting and the total probiotic cell count incorporated before pelleting was 10^6 CFU/g. The growth performance results (0-21 days) showed that AB and SYN groups had better weight gain than that of CT group ($p<0.05$), as well as in feed conversion rate (FCR)($p<0.05$), however there were no significantly differences among groups during 21-35 days. The performance efficiency factor (PEF) was 362.7(CT group), 423.5 (AB group), 368.1 (CM group), and 386.9 (SYN group), respectively ($p<0.05$). In conclusion, probiotic pelleted with could improve the growth performance and acts as an alternative to antibiotic growth promoter in this study.

Keywords: probiotic, broiler, pelleted feed, growth performance.

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Microbiology, Chemical and Sensory Characteristics of Cocoa Powder: The Effect of *Lactobacillus plantarum* HL 15 As Culture Starter and Fermentation Box Variation

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ABSTRACT

Cocoa powder is made from fermented cocoa beans which separated from fat and then ground and sifted to produce powder. Fermentation stage is an important stage in processing cocoa beans for chocolate flavor formation. The traditional fermentation done by farmer triggers fungal growth can produce toxin, called mycotoxin. The use of lactic acid bacteria in cocoa beans fermentation has been known to inhibit the growth of mycotoxin-producing fungi. The addition of *Lactobacillus plantarum* HL 15 as culture starter has known as antifungal. The objective of this research are to study the effect of *Lactobacillus plantarum* HL 15 as culture starter and fermentation box variation on microbiology, chemical and sensory characteristics of cocoa powder. Cocoa beans fermentation were conducted by starter culture addition and without starter culture addition and then using new fermentation box and old fermentation box. The analysis of cocoa powder was carried out include water content, pH, fungi contamination, and sensory evaluation on color, flavor and taste of cocoa powder. The result shown that water content (3.85 % to 4.55%), pH (4.85 to 4.95), and sensory evaluation is not significant differences for all treatment. The addition of *Lactobacillus plantarum* HL 15 as culture starter in old and new fermentation box has a smaller lever of fungi contamination compare to the treatment without *Lactobacillus plantarum* HL 15 as culture starter.

Keywords: *Lactobacillus plantarum* as culture starter, cocoa fermentation, fermentation box, cocoa powder.

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Viability and Antifungal Activity of *Lactobacillus plantarum* HL-15 Oven Dried Culture During Storage

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ABSTRACT

Cocoa is one of the plantation commodities that have high potential in Indonesia. But the quality of cocoa beans from Indonesia is still low, so the selling price of cocoa beans is low. One reason is that there is still a lot of microbiological contamination from fungi that can produce mycotoxins (Ochratoxin). Lactobacillus plantarum HL-15 is known to be able to inhibit the growth of mycotoxin producing fungi. To support the application of that culture, the research aims to find out the viability of oven dry starter using rice matrix and tapioca during 2 months of storage. Production of dried culture was started by fermentation of *L. plantarum* HL-15 then centrifuged to get the pellet. The pellet was mixed with filler (rice flour or tapioca) with a ratio pellet: filler (10%) = 1:1 (v/v) then dried. Drying machine used in this research was oven dryer with temperature 45°C for 20 hours. Dried culture was packaged in aluminium foil and sealed then stored at 4°C. Result showed that dry starter of Lactobacillus plantarum HL 15 tapioca matrix after drying has a viability of 8.94 log CFU / g while for rice matrix <4log CFU / g. During 2 months storage, starter viability decreases every month, until the second month the starter viability of the tapioca matrix drops to 6.07log CFU / g while for the rice matrix <2log CFU / g.

Keywords: viability, antifungal, oven dried culture, *Lactobacillus plantarum* HL 15

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Isolation of Lactic Acid Bacteria Capable of Cancer Cell Growth Inhibition from Thai Local Fermented Foods

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ABSTRACT

Lactic acid bacteria (LAB) were isolated from the Northeastern Thai fermented foods, which were Hua-hom-dhong (pickled onion), Pla-rah (pickled fish), Nham (sour pork or beef). Total of 88 isolates of bacteria which showed the clear zone around the colony on the $CaCO_3$ supplemented MRS medium were obtained and tested for their lactic acid bacteria properties, which were Gram staining, enzyme catalase test and lactose fermentation. These obtained LAB strains were further tested for their anticancer cell growth ability by adding cells or supernatants into CaCo 2 cell cultures. The results of MTT assay exhibited that all of the supernatants from all of the obtained LAB isolates could inhibit the colon cancer cell growth from 39 – 91%. On the other hand, only cells of 67 isolates of the obtained LAB could inhibit the cancer cell growth from 3 – 70%. Interestingly, some isolates of LAB could increase the cancer cell growth, such as the isolate SP-LP (12) showed the OD of MTT assay higher that the control (more than 100%). The isolates which showed the highest and the lowest cancer cell growth inhibitions, which were TH-R8 (5) and SP-LP (12) respectively, were also tested for their ability in inhibition of pathogenic bacterial growth. Results showed that the isolate TH-R8 (5) could inhibit *Salmonella Enteritidis*, *Sal. typhimureum* TISTR 292, *Escherichia coli* TISTR 780, *Staphylococcus aureus* TISTR 1466 and *Bacillus cereus* TISTR 678. On the other hands, the isolate SP-LP (12) could inhibit *Pseudomonas aerugenosa* and *Enterobacter aerogenes* TISTR 1540 only. However, these results have to be studied further.

Keywords: Anticancer cell growth, Northeastern Thai fermented food, CaCo 2 cell

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Molecular Detection of Antibiotics Resistant-Encoded Genes of *Lactobacillus rhamnosus* SMM strains

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ABSTRACT

Lactic acid bacteria, including *Lactobacillus* are gut normal flora that maintain homeostasis of human gastrointestinal tract. Many studies have investigated the role of *Lactobacillus* isolated from fermented product or milk as probiotics candidates. Potency of isolated *Lactobacillus* is strain-specific, including their resistance traits to antibiotics. There are two mechanisms of antibiotics resistance transfer including vertical and horizontal resistance transfers. Vertical transfer is chromosomally (DNA genome) encoded, while horizontal transfer is usually plasmid-encoded. Previous study of our group found that *Lactobacillus rhamnosus* isolated from mare milk (SMM-37, 49 and 58 strains) showed probiotics potency. By using modified-disk diffusion method, these isolates showed resistance to beta lactam, macrolide (erythromycin) and vancomycin, however have not been determined the encoded gene. Therefore, aims of this study were to determine antibiotics-encoded genes of *L. rhamnosus* SMM 37-2, 49 and 58 strains. Genomic DNA and plasmid isolation were performed and used as templates of PCR. Specific primers for amplifying *bla* (beta lactamase), *vanA, vanE, vanH* and *mefA* (erythromycin resistant) genes were used in this study. The results showed that none of genomic DNA was positive, while from plasmid isolation showed that all strains were positive for *bla, vanA, vanE* genes. One strain, SMM 58 showed *vanH* gene positive from plasmid. All strains were negative for Erythromicin resistant-gene. It suggests that

among *L. rhamnosus* strains showed different resistance genotype pattern (*strain-specific*). This data is baseline data for safety assessment as probiotic candidates.

Keywords: Lactobacillus rhamnosus, strain specific, mare milk, antibiotic resistant, genotype

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Antagonistic Effects of Fermented Soybean Meal as a By-product on the Growth of *Streptococcus agalactiae* Bovine Mastitis Pathogens

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ABSTRACT

Mastitis results in substantial problems in terms of animal welfare, food safety, and profitability of milk product. The most frequently isolated pathogens associated with clinical mastitis in Thailand are *Streptococcus agalactiae* and *Staphylococcus aureus*. The bacterium *Lactobacillus plantarum* is widely used in food production and in medical applications and is considered safe for human and animal use. This study investigated the antagonistic effects of fermented soybean meal (SBM) as a by-product on the growth of *S. agalactiae* bovine mastitis pathogen aiming to develop new approaches for improving silage quality and prevention mastitis disease. The culture condition of *L. plantarum* KL1 in 25% and 100% (w/v) SBM was also determined. The result showed that 100% (w/v) SBM showed the maximum *L. plantarum* KL1 growth with the amount of cell at 10^9 CFU/g SBM. The pH value decreased from 6.12 to 4.82 during fermentation and total acidity (TA) increased from 1% to 2%. This strain has on the ability to utilize the 25% and 100% (w/v) SBM as a source of carbohydrate and nutrient for growth. In bacteria growth inhibition, the growth of *S. agalactiae* (100%) was inhibited when it was incubated in the presence of *L. plantarum* KL1. The amount of *S. agalactiae* decrease of between 10^6 CFU/g and 0 CFU/g in co-culture when was compared with SBM that added only *S agalactiae* in fermentation process. The pH value decreased from 5. 62 to 5.00, and TA increased from 0.05% to 0.07%. It found to produce lactic acid, acetic acid, and propionic acid. The fermented SBM showed antagonistic activity against *S. agalactiae* bovine mastitis pathogen. It can be concluded that LAB tested

in this study can be used as silage inoculants for controlling pathogens in the silo which may contaminate to environment and cause of mastitis in dairy cow.

Keywords: lactic acid bacteria, antimicrobial agent, bovine mastitis pathogen, soybean meal

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The Level of Aerobics In The Production Of Black Glutinous Rice Tape And Its Potential As A Probiotic Food

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ABSTRACT

The level of aerobics is one of the environmental factors that determine the success of microbial growth in producing glutinous rice tape. This study aims to determine the appropriate aerobic level for making black glutinous tape as a probiotic food and drink. The research was done as follows: black glutinous rice was washed, soaked, steamed, cooled, and inoculated with MK 'ragi ' (inoculum) of 0.2%. Fermentation was carried out with 40 hours of aerobic, semi-aerobic and anaerobic conditions, followed by 24 hours anaerobic fermentation. Analysis carried out on black rice tape includes levels of starch, reducing sugars, ethanol, and lactic acid, the number of lactic acid bacteria, and organoleptic tests. Analysis carried out on black rice tape liquid includes dissolved solids total, a number of lactic acid bacteria, and organoleptic test. The results showed that the suitable aerobic level conditions for producing black glutinous rice tape as probiotic food and drink were fermented aerobically for 48 hours, and continued for 24 hours anaerobic fermentation. Black glutinous tape produced from the treatment was favored by panelists and had a starch of 27.06% (db), reducing sugars of 30.915% (db), ethanol of 11.73% (wb), lactic acid of 0.11% (wb) and the number of lactic acid bacteria of 2.4 x 107 cfu/g. Black glutinous tape liquid with aerobic treatment contained lactic acid bacteria of 3.9 x 108 cfu/ml and dissolved solids total of 37.63% (wb). The black glutinous tape and liquid have the potential as a probiotic food and drink.

Keywords: aerobics, black glutinous rice, probiotic, tape

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Optimization of Exopolysaccharide Production By *Lactobacillus casei* AL.15

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ABSTRACT

Exopolysaccharide (EPS) was one of polysaccharide produced from microorganisms. This polysaccharide is usually produced by lactic acid bacteria, and has a very large usability for food products and pharmaceutical products. Exopolysaccharide also very useful for stabilizer, emulsifier, gelling agent and have a good capability to bind liquid (water). Optimization of production exopolysaccharide produced by *Lactobacillus casei* AL.15 determined using Response Surface Method (RSM), with three different parameters namely temperature, incubation time and the amount of glucose in the media *de Mann Rogosa Shape broth* (MRSB). Response surface method is applied using a central composite design consisting of 20 repetitions at 15 unique combinations plus 5 replicates at one combinations. *Lactobacillus casei* AL.15 grown in 750 ml MRSB media, capable of producing EPS of 891.26 mg / L, with a potential dominant at 37-42°C temperature, growth time 24-48 hours and glucose 10-11.8%. EPS production optimization occurs on level -1; -1; 0 the temperature 42°C, 48 hours of incubation and 10% addition of glucose. *Lactobacillus casei* AL.15 have a pretty good ability in producing exopolysaccharide in temperature, time and amount of glucose is obtained, the actually yield under this conditions was 606,03 mg of exopolysaccharide/ liter. This method could be used to produce exopolysaccharide in large numbers to then analyzed the characteristics of the resulting ekspolisakarida.

Keywords: Optimization, Exopolysaccharide, *Lactobacillus casei* AL.15

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Metagenomics Study Revealed That Oncom, A Peanut Press-Cake Fermentation Food, Is A Promising Source Of Lactic Acid Bacteria

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ABSTRACT

Oncom is known as an Indonesian fermented food made from fermentation of a peanut press-cake by fungus like *Neurospora* spp. and *Rhizopus oligosporus*. Beside *Neurospora* and *Rhizopus* as the main fermenter, bacteria may also play a role in fermentation process. However, the bacterial community that living in oncom is not well described. So that, this study was aimed to explore bacterial community living in oncom, especially lactic acid bacteria group. The 16S metagenomics sequencing was applied to investigate bacterial diversity in red oncom and black oncom. Shotgun metagenomics was used to study bacterial diversity and functionality in oncom Bandung. Based on 16S-metagomocis analyses, the results showed that lactic acid was found in all six oncom samples (3 red oncom and 3 black oncom). Order Lactobacillale was found 20-70% of total bacteria in oncom. Deeper analyses by using shotgun metagenomics analyses also found that lactic acid was predominantly inhabited oncom Bandung. *Lactobacillus*, *Weissella*, *Leuconostoc*, were a member of lactic acid bacteria group found in three oncom Bandung samples. This study showed that oncom is one of the promising sources of lactic acid bacteria. Further study should be conducted to understand the role of lactic acid bacteria in oncom fermentation process.

Keywords: Oncom, Lactic Acid Bacteria, 16S-rDNA, Shotgun Metagenomics.

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Angiotensin Converting Enzym Inhibitory (Ace-I) In Fermented Soy Milk by Indigenous Lactic Acid Bacteria

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ABSTRACT

Soybean is one source of vegetable protein which can be utilized for the production of bioactive antihypertensive peptides. A study was conducted to investigate the potential of different lactic acid bacteria cultures to release bioactive antihypertensive peptides in soy milk through inhibition of angiotensin converting enzyme (ACE). A total of 10 LAB isolated from fermented foods (tempeh and kefir) and non-fermented foods (breast milk) were grown in reconstituted skim milk (11%) and incubated at 37^{0}C until pH 4.6 reached. Evaluation was carried out for LAB count, lactic acid concentration, peptide content and ACE inhibitory activity. The results showed that all isolate grew well in milk. The LAB population ranging from 8.98 ± 0.11 log CFU/mL to 9.62 ± 0.23 log CFU/mL with the fermentation time different between cultures ranging from 24 to 48 hours. The lactic acid produced ranged from 0.66 ± 0.03 % to 0.86 ± 0.06 %, whereas the total protein content ranged from 0.32 ± 0.03 mg/mL to 0.44 ± 0.01 mg/mL with the amount of peptides ranged from 2.86 ± 0.12 mg/mL to 4.68 ± 0.18 mg/mL. The highest of angiotensin converting enzyme inhibitory activities were found in soy milk inoculated with *Lb. fermentum* R6.

Keywords: antihypertensive bioactive peptides, fermented soy milk, ACE inhibitory activity, lactic acid bacteria, *Lb. fermentum*

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In-Vitro and In-Vivo Evaluation of The Probiotic Potential of *Lactobacillus paracasei* Strain K56 FOR Modulating Gastrointestinal Health

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ABSTRACT

Lactobacillus paracasei strain K56, a probiotic isolated from a healthy Chinese child's microbiota, was evaluated for its tolerance to the gastrointestinal environment, adhesion properties and impact on the intestinal flora composition. The survival rates of K56 were 84.31% and 82.73% after treatment for 1h in pH 3.0 medium, and 0.5 g/100mL bile salt medium, respectively. Adhesion of the strain to Caco-2 cells was assessed and indicated that K56 is likely to strongly interact with the intestinal epithelia of humans. The inhibition of intestinal pathogens by K56 was assessed using the Oxford Cup method and representatives of five potentially pathogenic bacterial species, including: *Escherichia coli; Candida albicans; Staphylococcus aureus; Klebsiella pneumoniae;* and *Streptococcus mutans;* were strongly inhibited. An in-vivo study was carried out to assess the effect of K56 on the intestinal microbiota in a mouse model. Balb/c male mice were randomly allocated to a blank group, model group, or a *L. paracasei* K56 group (2×10^9 CFU/d, human body dose). After 14 days intervention, the *L. paracasei* K56 group had significantly increased relative abundances of *Bifidobacteria* and *Lactobacillus* ($p < 0.05$) compared to the control groups, but had no significant effect on *Enterobacter, Enterococcus*, and *Clostridium perfringens* abundance. Conclusion: *L. paracasei* K56 is able to tolerate the intestinal environment, adhere to intestinal cells, inhibit intestinal pathogens, and in-vivo, exerts a regulatory effect on the intestinal microflora in mice. Based on these findings, *L. paracasei* strain K56 has strong potential as a probiotic for application in the treatment or prevention of intestinal diseases in humans.

Keywords: *Lactobacillus paracasei* strain K56, intestinal cell adhesion; inhibition of intestinal pathogens, probiotic.

CURRICULUM VITAE

CURRICULUM VITAE OF INVITED SPEAKERS

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Prof. Dr. Demberel SH

- Academician of Mongolian Academy of Science and Professor Veterinary medicine at the Mongolian University of Life Science in Ulaanbaatar, Mongolia
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- Professor in Food Micobiology, Faculty of Agricultural Technology, Universitas Gadjah Mada
- In 5 years, she published 45 journal papers such as: Gut Microbiota Profile in Healthy Indonesians, Development of the Traditional Tape Ketan into Probiotic Drink, Screening of lactic acid bacteria producing uricase and stability assessment in simulated gastrointestinal conditions
- Chairman of the Center for Food and Nutrition Studies, Universitas Gadjah Mada. Phone: 08122690013
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Prof. Dr. Endang S Rahayu , MS

Prof. Fransisco B. Elegado

- Research Professor & Program Leader of the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB).
- Researches on the functionalities of Philippine fermented foods, utilization of lactic acid bacteria and superior yeast for the production of improved starter cultures/ functional food products, process development in ethanol fermentation
- He is one of the assignees of the patent on a Detection Process for *Salmonella* and the specific Nucleotide sequence (Patent No. 1-2003-000241).

Dr. G. Balakrish Nair

- He is executive Director of Translational Health Science & Technology Institute (THSTI). India
- Director of Laboratory Science Division at the International Center for Diarrhoel Diseases Research, Dhaka, Bangladesh
- Research Interest: Immunology and Microbiology

Prof. Dr. Guo-Qing He

- Dr. HE Guoqing, professor for food microbiology at the Zhejiang University.
- He has served as dean of food science department at Zhejiang Agricultural University, vice dean of the college of biosystems engineering and food science at Zhejiang University
- President of Lactic Acid Bacteria Society of CIFST
- Now, he serves as vice-president of Asian Federation of Societies for Lactic Acid Bacteria

Prof.Dr. Hooi-Ling Foo

- Professor at Universiti Putra Malaysia (UPM).
- **Research interests**: purification and characterisation of enzymes; application of lactic acid bacteria and bioprocess development.
- Executive Board member of Asian Federation Societies of Lactic Acid Bacteria since 2009. She also has a long contribution record for Malaysian Society for Microbiology (MSM)

I Nengah Sujaya, Ph.D

- Lecturer at School of Public Health, Fact Mediciene, Udayana University, Bali
- Field of research: Food microbiology, Gut microbiota, Microbial ecology and systematics
- Current research: Development of Prebiotic and Probiotic from Indonesian Biodiversities

Prof. Ingrid S.Surono, MSC, PhD

- President of Indonesian Scientific Society Probiotics & Prebiotics (ISSPP)
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Dr. Jin-zhong Xiao

- Vice President of Japanese Society of Lactic Acid Bacteria.
- He has carried out many projects in the basic and functional researches of bifidobacteria and the development of yogurt at R&D center and published over 100 articles in the related area. (2015)
- **Current Research**: Microbiota and health, basic and functional aspects of probiotic bifidobacteria

Dr. Jiro Nakayama

- Professor at Departement of Bioscience and Biotechnology Faculty of Agriculture, Kyushu University, Japan
- The Japan Bioscience, Biotechnology, and Agrochemistry Society Awards for the encouragement of young scientists. "Bio-organic chemical and molecular biological studies on pheromone signaling in *Enterococcus faecalis*"
- Research topics: Human GI-tract microbiota (join Asian Microbiome Project)

Dr. Julie D Tan

- She is founding and first President of the Philippine Society for Lactic Acid Bacteria (PSLAB).
- Professor at the Visayas State University (VSU), Baybay City Leyte, Philippines.
- Field of specialization is Food Science focusing on applied microbiology and rootcrop processing.

Prof. Jyoti Prakash Tamang

- Dean of School of Life Sciences, Sikkim University India
- Known for : Studies on fermented food, identified the probiotic and functional properties of fermented food found in the Himalayan region and has studies its microbial diversity
- **Areas of Specialization** : Microbiology of fermented foods and beverages for last 32 years focusing on culture-dependent and culture-independent techniques of microbial diversity including metegenomics, functionality and health-promoting benefits.
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Prof. Dr. Kenji Sonomoto

- He was leading the Laboratory of Microbial Technology, Division of Systems Bioengineering at the Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University (Japan).
- Field(s) of specialization are: applied microbiology, enzyme engineering and genetic engineering.
- He is membership of Japan Society for Lactic Acid Bacteria (President; 2015 –)

Ken Ichiro Suzuki

- Professor, Dept Fermentation Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, Tokyo
- Award of Japan Society for Culture Collections "Establishment of a new microbial resource center and the advanced studies on the taxonomy of actinobacteria" (2009)
- SAJ Award (by the Society for Actinomycetes, Japan) "Chemotaxonomic study of Actinomycetes and Biological Resource Center"
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- Research interests: Molecular ecology of microbial communities in human intestine and fermented foods, Microbial sytematics on lactic acid bacteria and bifidobacteria, Culture Collection.
- His career at adviser of Japan Society for Lactic Acid Bacteria, and IEBC Observer of AFSLAB, Member of IUMS Subcommittee on the Taxonomy of *Bifidobacterium, Lactobacillus*

Prof. Maryam T Ebrahimi

- Associated Professor, Department of Biology, Science Faculty IAU, Central Tehran Branch
- Chair of Iranian Society of Probiotic and Functional Food (ISPFF)
- Cooperation with Agriculture Biotechnology Research Institute of Iran
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Dr. Massalin Nakphaichit

- Academic staff in research unit of probiotics and prebiotics for health, department of Biotechnology, faculty of Agro-Industry, Kasetsart University, Thailand.
- Her research group is involved the effect of probiotic on health, biomarker identification, prebiotic production from agricultural waste and probiotic encapsulation.
- Interested in impact of functional food including probiotic, prebiotic and synbiotic on human and chicken gut microbiota.
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- Bioprocess Technology, School of Industrial Technology Universiti Sains Malaysia, Malaysia
- IAP Young Scientist, to represent Malaysia at the World Science Forum in Budapest to pitch for changes in science policies in the Hungarian Parliament (2015)
- Research Interest: Probiotics for Skin Health

Prof. Dr. Min-Tze Liong

Prof. Ming-Ju Chen

- Professor at the University of National Taiwan University (NTU), Taiwan. AT NTU, she has served as the director of Center for International Agricultural Education and Academic Exchanges.
- Research interests: isolation and identification of new bacteria and yeasts from different resources and application these strains in human food and animal feed.
- Currently, she serves as Presidents of the Association of Animal Science and Taiwan Association of Lactic Acid Bacteria

Dr. Neerja Hajela

- Get her masters in Microbiology and Ph.D in Biotechnology
- Involved in the development, planning, and implementation of scientific programmes including clinical trials for company. She is associated with the Asian Federation of Societies for Lactic Acid Bacteria (AFSLAB)
- She is currently the Head of Science and Regulatory Affairs at Yakult Danone India Pvt. Ltd.
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Prof. Park Yong-Ha, Ph.D

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- Founder of Probiotic Institute and Probiotic House
- Specialities : Biotechnology, Immunology, and Microbiology
- Acclaimed authority on lactobacillus research for over 30 years, and reported 'Lactobacillus kimchii' for the first time in the world to the international academic circles to establish the position of Korea as the home of kimchi

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Prof. Sung Shik Yoon

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- His project is Bacteriosin production from lactic acid bacteria
- He has published 18 research paper
- Skills and Expertise: lactic acid bacteria, fermentation, bacteriophage, DNA, *Lactobacillus,* milk

Prof. Tadao Saito

- Professor, Graduate School of Agricultural Sciences, Laboratory od Animal Products Chemistry, Division of Bioscience and Biotechnology for Future Bioindustries
- Japanese Dairy Science Association (representative manager, secretary of editorial committee)
- Japanese Society for Lactic Acid Bacteria (JSLAB) (director)
- Japanese Animal Science Association (officer)

Takuya Akiyama

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Prof. Dr. Wilhelm Heinrich Holzapfel

- Chair Professor, Handong Global University, Pohang, South Korea
- Around 45 years' experience in teaching and research in basic Microbiology, and various fields of Applied Microbiology, including Industrial Microbiology, Food Microbiology and Food Hygiene
- **Major Research Areas:** Lactic acid bacteria (focus on: taxonomy, diversity, ecology, functionality, applications, safety), probiotics, gut microbiota, food microbiology, food biotechnology.

Dr. Yantyati Widyastuti

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- Her research is identification and characterization lactic acid bacteria, and the utilization of lactic acid bacteria to silase inokulan and probiotic for cow
- Has published 58 research papers
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Prof. Ying-Chieh Tsai

- Distinguished professor at the Institute of Biochemistry and Molecular Biology at National Yang Ming University since 1983
- Founded the Taiwan Association of Lactic Acid Bacteria, in 2002 and served as President until 2006
- Currently, Professor Tsai is conducting ground-breaking research on the microbiota-gut brain axis, specifically pioneering development of psychobiotics that he explores their commercial and health benefits.
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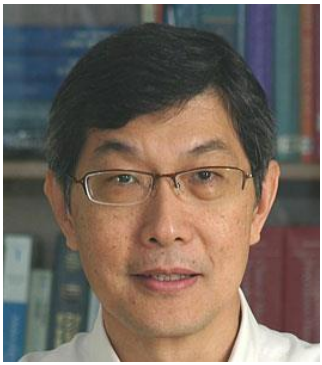
Prof.Yuan Kun Lee



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10th Asian Conference on Lactic Acid Bacteria
Yogyakarta, August 29-31, 2019

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