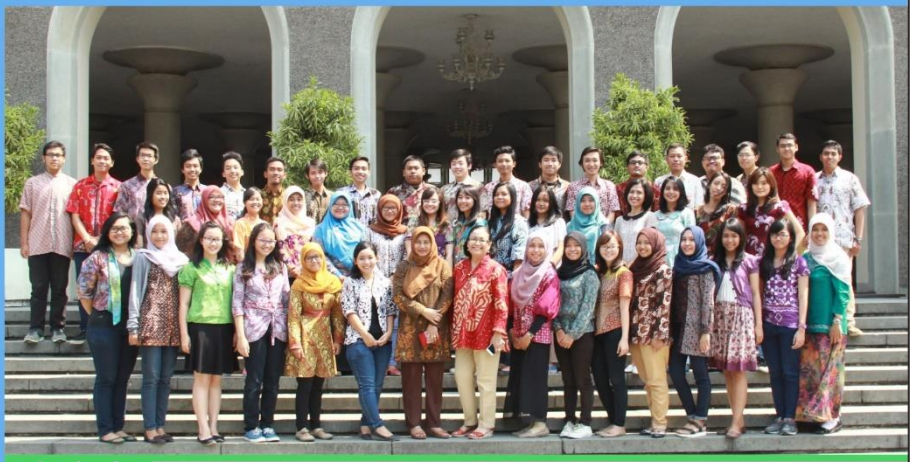
The 5th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (5th IC-ISLAB-GM)



Program Book

Better Life with Lactic Acid Bacteria
Exploring Novel Functions of Lactic Acid Bacteria
and Exploring Gut Microbiota

November, 13-14th 2015

Kamarijani-Soenjoto Auditorium
Faculty of Agricultural Technology
Universitas Gadjah Mada
Yogyakarta

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(The 5th IC-ISLAB-GM)

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INTRODUCTION

Indonesia as a mega-diversity country has diverse microorganisms, including lactic acid bacteria. These bacteria which have varied physiological functions have been isolated and investigated associated with the benefit of human life. The utilizations of lactic acid bacteria expand into many areas of food, health, and industries. Lactic acid bacteria play many roles in traditional Indonesian fermented foods such as *tape, kecap,* and *asinan*. Many species and strains of lactic acid bacteria have been suggested to have many beneficial effects on the health of the digestive tract of humans. Many strains of lactic acid bacteria have been applied into probiotic products. Administration of specific strains of lactobacilli and/or *bifidobacteria* was found to be effective in the treatment/prevention of rotavirus, antibiotic-associated, and pathogenic diarrhea. The ability of specific probiotics to enhance immune function in infant has also been reported.

Research has been carried related to the development of science and technology in microbiological area. Lactic acid bacteria could be explored for novel function, particularly to support the health benefit for human being and other life. To support the preservation of potential microorganisms, culture collection should be managed in a good management system. Therefore, it is necessary to disseminate these research findings and experiences as well as how to manage culture collection among researcher, pediatrician, students, industries and other stakeholders. The objectives of this conference are to disseminate the research achievement among the researchers, to explore novel functions of lactic acid bacteria, and to strengthen the network among the international and national researchers as well as industrial partner.

The conference will be organized by the Indonesian Society for Lactic Acid Bacteria (ISLAB) and the Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia in cooperation with the Indonesian Society for Microbiology (PERMI) and PT. Yakult Indonesia Persada. Various speakers from inside and outside the country those have expertise in this field will be present as the main speakers. It is expected that the seminar will be attended by researchers, lecturers, doctors, students, industrial society, from local and abroad.

PREFACE

Chairman of Steering Committee
By Tyas Utami

It is my great pleasure to welcome you to "The 5th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (5th IC-ISLAB-GM)". My warmest greeting to the honorable delegates, guests and all participants and wish you all have very good time while in Yogyakarta.

The 5th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (5th IC-ISLAB-GM), will be held on 13th-14th November 2015 at The Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta. The objectives of the meeting are to disseminate the recent research achievements in lactic acid bacteria, to explore the role, benefit and novel function of lactic acid bacteria; and to strengthen the network among the national and international universities, research institutes, government agencies, and industries in exploring the role and application of lactic acid bacteria and gut microbiota.

The participants of this conference come from universities, research institute, government agencies and industries from Indonesia and overseas. We have invited important speakers from Japan, Finland, Taiwan, Philippines, Denmark, Thailand and Indonesia. We also have presentation from industry (Yakult). There are two plenary lectures, two parallel session for technical oral presentation, and poster presentation in the first day of conference. We wish that The 5th ISLAB to be an opportunity not only for sharing ideas and experiences but also for establishing, and extending friendship among scientists and strengthening your personal and professional networks. For this time, the proceeding of the 5th IC-ISLAB-GM will be publish by Knowledge Publisher as the first proceeding to be publish online. We are pleased to inform you that there will be the 1st ISLAB-GM congress tonight. The organizing committee invites all the participants to attend dinner and cultural night this evening in this room. There will be an excursion to a famous Borobudur Temple on Saturday morning.

I would like to thank to all presenters and participant for the tremendous effort and time spent in the conference. On behalf of the organizing committee, I would like to express my sincerely thanks to ISLAB member, Yakult company and the Faculty of Agricultural Technology for their support, facilities and other contribution for the success of this Conference. Finally, I would like to take this opportunity to thank to all the

colleagues, the steering committee and organizing committee for their never ending precious cooperation that made this event possible.

I wish you all enjoy The 5th ISLAB conference and the wonderful Yogyakarta.

Chairperson of Organizing Committee

Dr. Tyas Utami

PREFACE

Dean of the Faculty of Agricultural Technology, Universitas Gadjah Mada

By Lilik Sutiarso

Distinguished Guests, Ladies and Gentlemen, Participants of The 5th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota.

I would like to warmly welcome all of you to The 5th International Conference of Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (5thIC-ISLAB-GM) entitled *Better Life with Lactic Acid Bacteria: Exploring Novel Functions of Lactic Acid Bacteria and Exploring Gut Microbiota*, which is held from November 13-14, 2015 at Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

As we all know, lactic acid bacteria play many roles in traditional Indonesian fermented foods such as *tape, kecap, asinan,* and fermented fish. I believed that lactic acid bacteria are also play similar roles in the fermentation of many other country's foods, as well. Many researchers have isolated and characterized lactic acid bacteria from various sources, and found out that many strain of lactic acid bacteria have been suggested to have certain beneficial effects on food qualities as well as on human health. Therefore, it is necessary to disseminate these research findings and exchange knowledge and experience between researchers, industrial partners and universities.

Since the founding and further activities of the ISLAB are involved the role of academician, bussiness sector, government institution, and community (general or special profession). This involvement is certainly due to the important of building and maintaining the community networking, which is cannot be excluded in their competence and needs. Regarding to the several member of the ISLAB, the role of the academician and researcher is holding one of main functions in maintaining the scientific interest and activities. In other hands, the role of business sector and government are also in accordance to community outreach from the scientific activities for application and legal aspects.

I hope that this International meeting activity does not only increasing the collaboration research but also can improve the education value for nation and world. It is important that research activities and community outreach should be running in accordance to science

development which can strengthen the competence of higher education institutions.

Finally, I would like to express my sincere thanks to all participants, companies and other parties for the contributions and support to the conference. I wish all the participants an inspiring and fulfilling conference. Have a wonderful time in Yogyakarta, Indonesia.

Faculty of Agricultural Technology,
Universitas Gadjah Mada,
Dean,

Prof. Dr. Ir. Lilik Sutiarso, M. Eng

PREFACE

Chairperson of Indonesian Society for Lactic Acid Bacteria
By Endang S Rahayu

The Indonesian Society for Lactic Acid Bacteria was established at Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, 12 March 2003, just few months after the establishment of Asian Federation of Society for Lactic Acid Bacteria (AFSLAB) at Tokyo, November 2002. Objectives of ISLAB are: (1) enhancement of the interaction and facilitate the communication between Indonesian scientists, industries, communities who are interested in Lactic Acid Bacteria and related subjects through scientific meeting; (2) establishment of research-collaboration between members; (3) to promote the activity of ISLAB to international scientific community. As professional society, ISLAB has organized 5 scientific meeting including this time. The first meeting was organized at Bali in collaboration with the 3rd Asian Conference for Lactic Acid Bacteria (ACLAB) and Congress of the Indonesian Society for Microbiology (PERMI), 25-26 August 2005; after that, ISLAB conference was organized at this Faculty for every two years, and now is the 5th international conference of ISLAB.

One of the interesting research topic of lactic acid bacteria is associated with probiotics. Indonesian researchers have conducted research related with this topic for many years. Selected indigenous probiotic strains have been promoted for industrial use. Probiotic is believed to be important in supporting intestinal health, together with the indigenous gut microbiota. Lactic acid bacteria, probiotics, and gut microbiota are scientifically correlated each others. In Indonesia, research associated with gut microbiota have also been started. After long disscussion between the founders of Indonesian Society for Lactic Acid Bacteria (ISLAB) and several prominent Indonesian Researchers on gut microbiota, we have a conclusion to expand Indonesian Society for Lactic Acid Bactera (ISLAB) becoming Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (ISLAB-GM). Today we declare that Indonesian Society for Lactic Acid Bacteria (ISLAB) now is officially change into Indonesian Society for Lactic Acid Bacteria and Gut Microbiota (ISLAB-GM).

Without changing the tradition, since existence of ISLAB (for 12 years) is under PERMI, ISLAB-GM was also remain under its core organizations in the field of microbiology namely PERMI (Indonesian Society for Microbiology).

PROGRAM-5th IC-ISLAB

1st Day (November 13th, 2015)	
07.00 – 07.45	Registration, welcome drink and breakfast
07.45 – 08.00	Welcome dance
08.00 – 08.30	Opening ceremony
	Preface 1. Chairperson of Organizing Committee 2. Chairperson of Indonesian Society for Lactic Acid Bacteria 3. Dean Faculty of Agricultural Technology, Universitas Gadjah Mada
	Plenary Lectures I Moderator : Endang S. Rahayu and I Nengah Sujaya
08.30 – 09.00	Dennis Nielsen (Kobenhavns Universitet, Denmark) "The Role of Gut Microbiota in Health and Disease"
09.00 – 09.30	M. Juffrie (Universitas Gadjah Mada) "The Effect of Probiotic, Prebiotic on Mucosal Immune Response and Stunting"
09.30 – 10.00	Jiro Nakayama (Kyushu University) "Asian Microbiome Project toward Phase III: Investigation on The Link between Diet and Gut Microbiota"
10.00 – 10.30	Seppo Salminen (University of Turku, Finland) "Probiotics and Microbiota Programming: What Is Known on Probiotic Effects?"
10.30 – 11.00	Koichi Watanabe (National Taiwan university, Taiwan) "Enumeration of Viable Cells is a Required Procedure to Assess Beneficial Effects of Probiotics"
11.00 – 11.20	Tyas Utami (Universitas Gadjah Mada) "Effect of Consumption of Probiotics Drinks Towards Gut Enterobacteriaceae"

11.20 – 11.35	Yakult Presentation	
11.35 – 11.45	Photo Session	
11.45 – 14.00	Break, Poster Session and Lunch	
14.00 – 16.00	Parallel Session	
	Room A: Technical Session	Room B: Technical Session
	Plenary Lectures II Moderator : Yantyati Widyastuti and Nanik Suhartatik	
16.00 – 16.20	Puspita Lisdiyanti (LIPI, Indonesia)	
16.20 – 16.50	Julie D. Tan (Visayas State University, Phillipines) "Recent Studies on the Association of Lactic Acid Bacteria in Fermented Foods in the Philippines"	
16.50 – 17.10	Anil K. Anal (Asian Institute of Technology, Thailand) "Biopreservation by Lactic Acid Bacteria (LAB)"	
17.10 – 17.30	Ken Ichiro Suzuki (NBRC, Japan) "The Role of Culture Collections in The CBD Era"	
17.30 – 17.50	Agus Wijaya (Universitas Sriwijaya, Indonesia) "Is *Lactobacillus* The Dominant Genus of Lactic Acid Bacteriain Indonesian Indigenous Fermented Foods?"	
17.50 – 19.00	Break and Dinner	
19.00 – 20.00	Cultural Night	
20.00 – 21.00	The 1st ISLAB Congress	
2nd Day (November 14th, 2015)		
08.00 – till drop	Excursion (Borobudur Tour)	

TECHNICAL SESSION PROGRAMME

Time	Note	Speaker	Title
Room A Moderator : Siswa Setyahadi and Widodo			
14.00 – 14.16	A1	I Nengah Sujaya	Resistance of *Lactobacillus* sp F213 in Human Gastrointestinal Tract and Its Health Promoting Effects
14.16 – 14.29	A2	Afriza Yelnetty	Characteristics and Sensory Quality of Goat Milk Yogurt Using Sucrose With Different Levels and Starch from Red Kidney Bean (P*haseolus vulgaris, cv*) as Prebiotics Source
14.29 – 14.42	A3	Ahmad Nimatullah Al-Baarri	Lactic Acid Recovery by Fortification of Mangoes Fruit Extract Into Manufacture of Yogurt Drink and The Profile of Dried Yogurt During Storage
14.42 – 14.55	A4	Amelia Juwana	Probiotic and Antimicrobial Potential of Lactic Acid Bacteria Which Screened *Mandai* (Fermented *Dami of Cempedak* (*Artocarpus champeden Spreng*.))
14.55 – 15.08	A5	Hasyrul Hamzah	Isolation and Characterization of Lactic Acid Bacteria as Probiotics in Dangke and Tape
15.08 – 15.21	A6	Hazel Alena Diamante Tan	Efficacy of Alginate-Taro [*Colocasia esculenta* (L.) Schott] Starch Encapsulation of Starter Culture for Yoghurt Processing

15.21 – 15.34	A7	Iskandar Azmy	The Development of Indigenous Strain on Fermented Milk Product: A New Strain Combination
15.34 – 15.47	A8	Shinta Maharani	Effects of Temperature and Inoculum Concentration on Chemical and Microbiological Changes of Black Soybean Milk Yogurt
15.47 – 16.00	A9	Widodo	The Quality of Fermented Milk Produced Using Human-Origin Lactic Acid Bacteria as Starters
Room B Moderator : Achmad Dinoto and Lindayani			
14.00 – 14.16	B1	Achmad Dinoto	The Chicken Gut Microbiota : Lesson Learned from SATREPS Project
14.16 – 14.29	B2	Satriya Arbian	Isolation of Human Origin Methyl Mercury Resistant LAB from Sekotong, West Lombok, Indonesia
14.29 – 14.42	B3	Lindayani	Combination between Salt Concentration and Fermentation Temperature on Probiotic Capability and Antimicrobial Activity of Lactic Acid Bacteria from Isolation of Yellow Betung Bamboo Shoot Pickle
14.42 – 14.55	B4	Lorentia Santoso	*In Vitro* Detection of Bacteriocin Inhibitory Activity of *Lactobacillus sp.* Isolated from *Betung* Bamboo Shoot (*Dendrocalamus asper*)Pickles Under Different Fermentation Conditions and Medium Compositions

14.55 – 15.08	B5	Muthia Cita Hapsari	Adhesiveness and Microstructure of Rehydrated Avocado-Fortified-Yogurt
15.08 – 15.21	B6	Nanik Suhartatik	Biodegradation of Anthocyanin Using Beta Glukosidase from *Pediococcus pentosaceus* N11.16
15.21 – 15.34	B7	Rio Jati Kusuma	A Novel Class Nutrient, MicroRNA, Content in Dairy Food Products
15.34 – 15.47	B8	Tri Ardyati, Mafruhatun Ni'mah	Diversity of Lactic Acid Bacteria and Nutrition Content of "Yoguku" During Storage
15.47 – 16.00	B9	Yeanly Wuena Pinaria	Exopolysaccharide Producing Lactic Acid Bacteria Isolated from Palm Sap (*Arenga pinnata*)

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POSTER PRESENTER			
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2	Agustine Susilowati, Hakiki Melanie and Aspiyanto	Recovery of Fermented Inulin Fiber by Lactic Acid Bacteria (LAB) from Inulin Hydrolysate Using Inulinase Enzymes of *Scopulariopsis s*p.-CBS 1 and *Deuteromycetes s*p.-CBS4 as Cholesterol Binder	PP-2
3	Agustine Susilowati and Aspiyanto	Drying Process of Fermented Inulin Fiber Concentrate by *Bifidobacterium bifidum* as A Result of Concentration Process Using Ultrafiltration System as Dietary Fiber Source for Cholesterol Binder Instant Drink	PP-3
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INVITED SPEAKERS

The Chicken Gut Microbiota : Lesson Learned from SATREPS Project

Achmad Dinoto

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ABSTRACT

Studies on microbiology of chicken gut were intensively conducted within past ten years. The cummulative microbial surveys showed high abundance and diversity of bacteria in the chicken gastrointestinal tract. The information on microbial community was very important as the baseline data for the future exploration on microbial resources and for the improvement on productivity in the poultry industry. In Indonesia, under the project scheme of Science and Technology Research Partnership for Sustainable Development (SATREPS), a research collaboration between Indonesia and Japan were established, in which, one of research subjects was focussed on describing new taxa of chicken gut microbiota and screening of lactic acid bacteria for probiotic candidates. In this study both culture-dependent and –independent techniques were applied for the analysis of Indonesian chicken microbiota. As revealed by molecular analysis, less number of microbial phylotypes in chicken cecum were associated with already known species. This finding was in line with previously reported microbial surveys of the chicken gastrointestinal tract. In the culture-dependent study, several novel taxa of anaerobic *Bacteroides* derived from Indonesian chicken were succesfully described. However, most isolated lactic acid bacteria (LAB) were recognized to be known species belonging to the groups of *Lactobacillus* and *Enterococcus*. Concerning on the functional properties, several strains showed high growth inhibiting action against pathogenic strains and high tolerances on

the environmental barriers, thus they were indicated as potential probiotic candidates. As the project output, collection of well identified and characterized microorganisms of chicken gut will be deposited in Indonesian Culture Collection (InaCC), a national depository for microorganism, for the future utilization.

Keywords: gut microbiota, lactic acid bacteria, chicken, SATREPS project, Indonesian Culture Collection (InaCC)

Is *Lactobacillus* The Dominant Genus of Lactic a Acid Bacteria in Indonesian Indigenous Fermented Foods?

Agus Wijaya

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ABSTRACT

The Indonesian indigenous fermented foods were typically characterized by spontaneous fermentation. Salt was mostly used as selecting agent which enabled to inhibit the growth of both spoilage and pathogenic microorganisms during the process. There were plenty sorts of fermented foods from Indonesia as products of lactic acid bacteria (LAB) activities. They were categorized into the following groups: fermented fruits, vegetables, fishes, cassava tubers, rice and soybeans. The roles of LAB genera in the food groups were discussed. *Lactobacillus* was revealed as the the most important genus of LAB in Indonesian fermented foods, since it was observed in all fermented foods, and followed by *Pediococcus, Streptococcus, Leuconostoc, Enterococcus* and *Weissella.*

Keywords: *Lactobacillus*, lactic acid bacteria, Indonesian fermented foods

Biopreservation by Lactic Acid Bacteria (LAB)

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ABSTRACT

Food safety is an important issue worldwide. Number of people are directly affecting by foodborne infections and thus become a major public health concern. Emergence of new pathogen and recent epidemics of some poultry and other food-associated pathogen (bird flu, swine flu and cholera) created alarming situation for the food preservation and safety. Investigation of food borne pathogen presence in food is the prime interest of microbiologist and food technologist. A wide range of methods have been using for inactivation of food borne pathogen and food preservation. Interest is now rapidly diverting to use novel technologies such as preservation with the help of biomaterials and non-thermal technologies. Biopreservation is defined as the extension of shelf life and enhanced safety of foods by the use of natural or controlled microbiota and/or antimicrobial compounds, and has gained increasing attention in recent years. Consequently, certain lactic acid bacteria (LAB), with demonstrated antimicrobial properties commonly associated with foods, are being assayed to increase the safety and/or prolong the shelf life of foods. Biopreservation and bio-control by using lactic acid bacteria and bio-active edible coating for food preservation is an environmental and consumer friendly approach for the control of foodborne pathogens. The antagonistic properties of LAB derive from competition for nutrients and the production of one or more antimicrobial active metabolites such as organic acids (lactic and acetic), hydrogen peroxide, and antimicrobial peptides (bacteriocins). Nowadays the use of LAB bacteriocins is considered an integral part of hurdle technology. Their combined use allows most pathogenic and spoilage bacteria to be controlled and also extend their inhibitory activity spectrum to such intrinsically resistant organisms as the Gram-negative bacteria.

The Role of Gut Microbiota in Health and Disease

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ABSTRACT

The human gastro-intestinal (GI) tract is inhabited by a complex and dynamic consortium of trillions of microorganisms. During recent years it has become well established, that diseases like obesity and diabetes are interrelated to imbalances in gut microbiota (GM) composition. This has led to an increasing scientific, societal and commercial interest in understanding how a healthy GM can be maintained and possibly guided in a desired direction through external stimuli.

In the present talk a brief overview of the main methods used for GM characterisation including methods for studying "the less known" members of our gastrointestinal tract such as bacteriophages and eukaryotes will be given. Next, examples of how factors like birth mode, early GM colonization patterns, diet early and late in life and probiotics influence GM, later immunity, disease expression (with main focus on autoimmune diseases) and everyday life, will be given.

Resistance of *Lactobacillus* sp F213 in Human Gastrointestinal Tract and Its Health Promoting Effects

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ABSTRACT

Increasing death causing by noncommunicable diseases (NCD) is not only more common in modern societies but also in developing countries. This disease is thought associated with unhealthy life style. Probiotic offers opportunities in managing NCD and becoming popular world wide. This research was aimed to determine the resistance of *Lactobacillus* sp F213 (LbF213) in human gastrointestinal tract and its health promoting effects. Fifteen healthy human subjects participated in this study were administered with a capsule containing 7.5 x 10^8 CFU for 28 days. Fecal and blood samples were collected before, during and after 28 days administration. The population of lactic acid bacteria and anaerobes in fecal samples was enumerated by culture methods, while the LbF213 in fecal samples were detected using PCR-DGGE of fecal microbiomic DNA. Health promoting parameters such as lipid profile and TNF alfa were analyzed in blood samples.

The results showed that administration of 7.5 x 10^8 CFU for 4 weeks increased LAB population, 2.19X10^9 CFU /g before administration to 1.58x10^{10} after 28 days administration, while total anaerobe decreased from 4.47x10^{10} before administration to 1.78x10^{10} CFU /g after 28 days. *Lactobacillus* sp F213 was detected in fecal samples suggested that the Lb. F213 survived in the human GI and play role in modulation of human intestinal microbiota. The LbF213 altered lipid profile of human subjects, which likely to be subject dependent. The F213 reduced 6.29% cholesterol, 7.70% HDL and 8.54% LDL and increased 0.19% of TG after 28 days administration. The effect of LbF213 in lowering blood cholesterol was found to be higher in high blood cholesterol subjects compared to normal blood cholesterol subjects, 8.1% and 4.06%, respectively. Administration of F213 for 28 days lowered about 36% of TNF alfa titer in serum, 0.91

pg/dL before and 0.59 pg/dL after adinistration. Those results demosntrated that LbF213 resisted in human GI, slightly modify human normal intestinal microbiota and excreted health promoting effects.

Keywords: *Lactobacillus* sp F213, intestinal microbiota, probiotic, cholesterol

Asian Microbiome Project toward Phase III: Investigation on The Link between Diet and Gut Microbiota

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ABSTRACT

Several hundred microbial species inhabit the human gastrointestinal (GI) tract and constitute a complex ecological community. Multiple factors, both intrinsic and extrinsic, affect the GI tract microbiota. Those identified include microbes acquired at birth, diet, host physiology, drug intake and disease. Eventually, the gut microbiota varies immensely between individuals. Recent progress in molecular ecology, notably development of next generation sequencers (NGS), has realized precise profiling of the human gut microbiota based on the sequenced-based systematic taxonomy. Despite the huge diversity of species-level gut microbial composition across individuals, the genus-level community structure characterized based on NGS dataset can be classified in three types, defined as "enterotypes". Further, enterotype classification can be realized in the microbiome dataset consisting of 100-fold more unique genes as compared to the human genome. It nicely demonstrates that the enterotype classification reflects functional differences of the gut microbial community as well as compositional differences.

Enterotypes are observed beyond cohorts and human races but often associated with dietary habits. Our Asian cohort study, called "Asian Microbiome Project (AMP)", has shown the existence of two enterotype-like microbiota reflecting dietary habit as well as country of residence (Phase I). Metagenomics study suggests that dietary resistant starch may reduce bile acid level in the large intestine and recruit the Prevotella-driven enterotype. Changes in gut microbiota with age, namely decrease of *Bifidobacterium* and increase of *Enterobacteriaceae*, are commonly observed over the Asian population, while the abundance of these two bacteria groups differs widely across individuals and countries (Phase 2). It is further interesting to clarify the cause and effect of these changes,

which will deepen our insight into the link between gut microbiota and host homeostasis.

In Phase III study of AMP, we aim to understand the link between diet and gut microbiota. As a preliminary study, we looked into the correlation between dietary records and gut microbiota in children living in urban and rural sites in Philippines. Dietary practice questionnaire was performed with stool sample collection and we found that the difference in their gut microbiota nicely correlates with difference in their dietary habit, suggesting that modern diet may drive enterotype shift in Philippine children. In a further study in Phase III, we are planning to collect metabolomics data as well as microbiome data to understand the intestinal environment as a matrix between food and gut microbial community.

Keywords: gut microbiota, Asian microbiome project, metagenomics

Recent Studies on the Association of Lactic Acid Bacteria in Fermented Foods in the Philippines

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ABSTRACT

The Philippines is endowed with various types of raw materials which are ideal for the production of fermented foods. Lactic acid bacteria (LAB) predominates in most of the fermented foods in the Philippines. This paper covers recent reports of various researchers in the Philippines who have generated an array of information on the types of LAB that are associated with fermented foods. The association of LAB with foods can either be naturally-grown or added as pure inoculants either single or mixed cultures. This paper provides information on the types of LAB present in specific substrate ranging from plants, meat, fish and dairy products. Investigations on health-promoting effects and medicinal roles of LAB in the human body and important antimicrobial compounds elaborated by LAB are also discussed. The effect of LAB on the growth and survival of aquatic animals is also presented. This papers also includes some studies on methods of stabilizing the growth and activity of LAB for effective use as starter cultures in food fermentation.

The Role of Culture Collections in The CBD Era

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ABSTRACT

The availability of microbial strains from public culture collections is an essential infrastructure for confirmation of the reproducibility of the results of publication as well as the further use of the microbial strains. Culture collection is a principal component for the prokaryote taxonomy. However, it is facing to important issues to be solved. (1) Availability of the type strains must be guaranteed by the culture collection. It also means the identity of the strain to that the depositor intends to deposit. The culture collection generally determines the 16S rRNA gene sequence to confirm the identity by comparing with the sequence determined by the depositor. If the deposited type strain does not show the characteristics same as the description of the paper, the species may lose the status. However, matching of the 16S rRNA gene sequence is only one of the minimum requirements for the certification. The number of deposits with incorrect cultures is higher than that we imagine and accounted for 5% of the deposit in a culture collection. MALDO-TOF MS has been recently developed for identification of microorganisms with the database. Its usefulness for differentiation at the level lower than species is expected for the quality management of a culture collection. (2) The other issue is for the accessibility of the type strain in compliance with the national laws and regulations under the Convention on Biological Diversity (CBD) and the Nagoya Protocol (NP). CBD mentions that states have the sovereign right to the biological resources originated from the country. Cross-border transfer of biological samples is to be done under the laws of the provider's country. A culture collection must check the authenticity of the deposit when it receives the culture including the terms and condition for distribution permitted by the national authority of the original country. I am involved in the project of establishment of Indonesian Culture Collection, InaCC at RCB-LIPI since 2011 to promote the microbial resources originated in Indonesia. InaCC is not only for taxonomy, but also

for application aiming the commercialization. The microbial strains deposited to InaCC are maintained in correct way and distributed based on the terms and conditions agreed with the depositors in compliance with Indonesian laws and regulations. InaCC is expected to be the infrastructure to facilitate microbiology of Indonesia and international cooperation.

Enumeration of Viable Cells is a Required Procedure to Assess Beneficial Effects of Probiotics

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ABSTRACT

Increasing awareness and interest in healthy lifestyles has led to the widespread use of probiotics in a variety of fermented milk products. Lactic acid bacteria and *bifidobacteria* are the most widely used probiotics whose impacts on diverse end points of human health have been evidenced in numerous reports.

In order to exert maximal beneficial effects on the host, probiotics should be able to live and proliferate in the intestines, thereby increasing the number of viable cells secreting beneficial factors. Therefore, it is critical to enumerate accurately the population of viable microbes in the finished products and express this information to the consumer on the product label.

Meanwhile, it is commonly admitted that most effects of probiotic are strain-specific and cannot be extended to other probiotics of the same genus or species. Therefore, the probiotic strain must be a taxonomically defined at the species and strain level. Further, since the basic requirement for probiotics to exert their expected positive effects is to be alive and flourishing in the gut, it is therefore indispensable to establish specific methods to identify and quantify them appropriately. Indeed, the methods which can separately enumerate probiotic cells into two states, "viable" and "dead", are immensely important to deeply understand the mechanisms by which probiotics exert beneficial effects in the gut and hence are also helpful in verifying the impact of probiotics on our health.

In this presentation, I will discuss not only the current situation and limitations with respect to the methods for the species/ strain identification of probiotics, but also the methods for precise enumeration of probiotics by using culture-dependent and alternative techniques to quantify viable cells.

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The effect of Probiotic, Prebiotic on Mucosal Immune Response and Stunting

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ABSTRACT

Probiotic and prebiotic are famous with name synbiotic have been recognized have multiple benefit according to many studies since hundred years ago. In Yogyakarta there are many kind local food that proofed has effect to healthy status. In the two studies below author present the remarkable results using a local food that almost every time as a daily food. *Lactobacillus plantarum* strain Muth 7 have been studied by some researcher and promote very nice response in immune in GI tract, *Lactobacillus plantarum* Dad 13 has same effect as well. In the study *of L. plantarum* Muth 7 has been founded that this probiotic has strong effect in innate and adaptive immune response in mucosa*. Lactobacillus plantarum* DAD 13 also has effect in enhance the absorption of calcium in intestinal in results to accelerate the linier growth of borne

Probiotics and Microbiota Programming: What Is Known on Probiotic Effects?

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ABSTRACT

Pregnancy and early infancy comprise the most critical stage for microbiota programming for later health. The key elements here include the mother's genetic background, environment and nutritional state as well as microbiome in the mother's gut and mucosal surfaces. Additional factors comprise of antibiotics administered either to the mother or the infant or both and exposure to other pharmaceuticals. The collective composition and the compositional development of the indigenous intestinal microbiota, co-evolves with the immune systems of the neonate (Rautava et al 2012; Koren et al 2012). Following delivery, human milk contains microbes, which can be mimicked by specific probiotics (Cabrera - Rubio, et al. 2012). Both previously mentioned influence programming.

The progress in human microbiome and probiotic research has created a new possitility to define effective strategies and treatment modalities to create a healthier microbiota. Well controlled human intervention trials and systematic reviews and meta-analyses continue to be reported and they provide already convincing evidence of the benefits of probiotics during pregnancy and lactation, with valuable public health implications. Also the term probiotic has been redefined (Hill, et al., 2014). Evidence-based recommendations demonstrate the benefits of specific strains of probiotics on infant health, including treatment and risk reduction of acute gastroenteritis and antibiotic associated disturbances. Several other areas appear promising for future applications. However, it is important to focus on probiotic strains, which have been proven effective. As each strain is different, results in infant studies cannot be extrapolated even to closely related strains.

The composition of the gut microbiota, and thus also the modification of the gut microbiota by specific probiotics or prebiotics early in life has an impact on the later disease risk. Probiotic effects have

been attributed to restoration to normal of increased intestinal permeability, improvement of the intestinal barrier functions, alleviation of the intestinal inflammation, and reduced generation of pro-inflammatory cytokines. Recent evidence from experimental and clinical studies indicates modifying gut microbiota is also associated with the control of body weight and energy metabolism and specific probiotics may assist in creating such control. Specific probiotics may also directly influence energy extraction from different dietary components and energy storage in the human body. Both functions can contribute to insulin resistance and the inflammatory state characterizing obesity. Thus, the focus of current research is on identifying and characterizing specific probiotic strains and food matrices to counteract detrimental microbiota deviations in mothers during pregnancy and infants during breastfeeding (Collado, et al., 2015). Such products will form the basis of future treatment and prevention modalities.

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The Effect of Consumption of Probiotic Drink on Gut *Enterobacteriaceae*

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ABSTRACTS

Many studies have supported the beneficial effects of probiotic for human health. The probiotic efficacy relies on their ability to survive in the digestive system and be able to proliferate in the gut. The ability of probiotic bacteria to survive in the gastrointestinal tract varies considerably between species. Factors such as type and composition of food consumed, life style, age, environment and race might have influence on survival of probiotic. The present of *Lactobacillus casei* strain Shirota (LcS) to the level of 6.6 ± 0.6 $\log_{10}$ CFU/g feces of healthy Indonesian volunteers after ingestion of fermented milk containing LcS indicated that LcS could survive and colonize in the gastrointestinal tract. It was found that the numbers of *Enterobacteriaceae, Escherichia coli* and *coliform* non *E.coli* in the feces decreased in almost half of the volunteers. *Lactobacillus plantarum* Dad 13, isolated from *dadih*, a traditional fermented buffalo milk in West Sumatera, Indonesia met the basic requirement as probiotic and had some functional properties. Study has been conducted to investigate the recovery of *L. plantarum* Dad 13 from the intestine of healthy Indonesian volunteers after consumption of fermented milk containing containing *L. plantarum* Dad 13 and its effect on gut *Enterobacteriaceae*. *Lactobacillus plantarum* cells in the feces were counted using selective agar medium (LPSM). The number of *L. plantarum* was increased significantly in all subject after consuming fermented milk containing *L. plantarum* Dad 13. Decreases of the number of fecal *Enterobacteriaceae, Escherichia coli* and *coliform* non *E.coli* were found in more than half of the volunteers. Based on BOX AIR - PCR analysis, *L. plantarum* Dad 13 was detected from volunteers's feces during

consumption period. It indicated that *L. plantarum* Dad 13 could survive in the digestive tract.

Keywords: probiotic, survival, gut *Enterobacteriaceae*, molecular detection

ORAL PRESENTERS

Characteristics and Sensory Quality of Goat Milk Yogurt Using Sucrose With Different Levels and Starch from Red Kidney Bean (P*haseolus vulgaris, cv*) as Prebiotics Source

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ABSTRACT

This study aimed to determine the effect of different levels of sucrose against chemical, microbiological, and sensory characteristic of goat's milk yogurt that used starch from Red Kidney beans as a source of prebiotics. Lactic acid bacteria used are *Streptococcus thermophillus, Lactobacillus bulgaricus*, and *Lactobacillus acidophillus* as probiotic bacteria. All of this bacteria used as starter culture. This experiment was carried out in a Completely Randomized Design (CRD) consisted five treatments with three replication. Data were analyzed by variance analysis. Significant difference treatment effects on variables measured were tested using honestly significant difference (HSD). At sucrose level of 0, 2, 4, 6, and 8%, there is no significant difference ($P > 0.05$) on the results of analysis on ash, protein, and fat content; but significant difference is noticeably ($P < 0.05$) on total lactic acid bacteria, pH, water content, crude fiber, and sugar reduction. Sensory test result indicate that goat's milk yogurt with 6% sucrose is the most preferred.

Keywords: goat milk yoghurt, culture starter, prebiotic chemical, microbiological, sensory

Lactic Acid Recovery by Fortification of Mangoes Fruit Extract into Manufacture of Yogurt Drink and The Profile of Dried Yogurt during Storage

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ABSTRACT

It has been known that the drying process for producing dried yogurt exhibited the reduction on the population of lactic acid bacteria. This research aimed at the utilization of mangoes fruit extract in order to prevent the reduction of lactic acid bacteria population in dried yogurt. This research used *Streptococcus thermophillus* and *Lactobacillus delbrueckii* sub sp. *bulgaricus* at the initial count of 6.31±0.33 log CFU/ml. The treatment used the amount of 3% (v/v) mangoes extract that was added to the milk before HTST pasteurization process. The incubation was applied at 37˚C for 4 hours to produce yogurt. The vacuum-controlled-chamber unit at 50˚C for 8 hours was used to produce final product of dried yogurt. This final product was then packed in transparent-vacuumed-plastic bag and stored at room temperature for 30 days. Population of lactic acid bacteria in mangoes-fortified yogurt (MF-Yo) and non-mangoes-fortified yogurt (NMF-Yo) were counted using total plate count. The final product was also tested at Lab color testing instrument using digital color meter software by Macintosh® to measure L* value. The results indicated that the number of total lactic acid bacteria in mangoes-fortified yogurt (MF-Yo) and non-mangoes-fortified yogurt (NMF-Yo) were 3.89±0.28 and 3.19±0.62 log CFU/ml, respectively, indicating the hindrance of lactic acid bacteria's depletion by mangoes extract after drying process. Thirty days of storage remarkably changed the L* value of MF-Yo while NMF-Yo showed the negligible value of L*. This result may

open the opportunity to preventthe lost of lactic acid bacteria in dried yogurt using fruit extract.

Keywords: lactic acid bacteria, L* value, mangoes extract, storage time, dried yogurt

Probiotic and Antimicrobial Potential of Lactic Acid Bacteria Isolated from *Mandai* (Fermented *Dami* of *Cempedak* (*Artocarpus champeden* Spreng)

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ABSTRACT

Nowadays, lactic acid bacteria (LAB) still become the most beneficial microorganism due to their probiotic potential. In Indonesia, fermented traditional foods such as *tempoyak*, *bekasam*, and *sayur asin* has been found to be the habitat of LAB with probiotic potential or producing antimicrobes. The traditional fermented food used in this study was *mandai* which made from fermentation of *cempedak* (*Artocarpus champeden* Spreng) *dami.* The aim of this study was to evaluate probiotic and antimicrobe potential of lactic acid bacteria (LAB) isolated from *mandai*. The method used in this study was divided into three parts: screening the genera, probiotic potential (acid tolerance test and bile salt tolerance test) and antimicrobial analysis. Based on the screening result, 41 isolates were found as *Lactobacillus* genera, that were able to survive under low pH (acid tolerance) and 17 from 41 isolates were able to survive under bile condition (bile salt tolerance). The antimicrobial analysis showed that 15 isolates were able to produce antimicrobial substances against three pathogenic bacteria *i.e. Escherichia coli, Listeria monocytogenes,* and *Salmonella typhimurium*. The potential isolates which have been proven to have probiotic and antimicrobe potential were selected for further species identification using DNA fingerprinting.

Keywords: lactic acid bacteria, *mandai*, probiotic, antimicrobial

Isolation and Characterization of Lactic Acid Bacteria as Probiotics in *Dangke* and *Tape*

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ABSTRACT

A research on isolation and characterization of lactic acid bacteria as probiotic in *Dangke* and *Tape* has been conducted with the aim to obtain and characterize lactic acid bacteria isolated from fermented foods of *Tape* and *Dangk*e (processed product from glutinous rice and cow or buffalo's milk fermentation, respectively). Microbial candidate as lactic acid bacteria and probiotic was isolated from *Dangke* and *Tape*. Characterization parameter of probiotic bacteria consists of colony morphology observation, Gram staining, and biochemical test. The biochemical test consist of endurance test for pH, bile salts, catalyst, motility, gelatin hydrolysis, citrate, carbohydrate fermentation, and Triple Sugar Ion Agar (TSIA) test. Four samples showed characteristics as probiotic which isolated from each *Dangke* and *Tape*. All isolates are Gram positive bacteria with rod-shaped, able to grow in the medium with pH of 2.5, and three of them were able to grow in the medium containing synthetic bile salt of 1% and 5%. Based on biochemical test, the 8 isolates were motile; and catalase test were found negative for all isolates. During the fermentation, gelatin test showed a positive result, while on the citrate and H_2S test, all isolates were found negative. The research results can be concluded that all isolates showed characteristics of lactic acid bacteria.

Keywords: lactic acid bacteria, *Dangke, Tape*

Efficacy of Alginate-Taro [*Colocasia esculenta* (L.) Schott] Starch Encapsulation of Starter Culture for Yoghurt Processing

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ABSTRACT

The study determined the viability of the lactic acid bacteria, *L. bulgaricus,* and *S. thermophilus*, in yogurt which were encapsulated in different levels of alginate-taro starch matrix. The physico-chemical properties of the reconstituted skim milk medium and the sensory qualities of resulting yogurt were also determined. The developed alginate-taro starch encapsulated, non-encapsulated and pure alginate encapsulated starter cultures were compared in terms of their activity in yogurt processing. The highest percentage of taro starch (1.5%) was found to retain a high viable cell count of 3.4 $x10^{10}$ colony forming unit (CFU/ml) after 12-day storage at 4°C. No significant effect of encapsulated starter cultures was observed on the physico-chemical properties (i.e. pH, TSS, and % lactic acid) of reconstituted skim milk. Incubation and storage periods significantly affected the physico-chemical properties of the reconstituted skim milk (p<0.05). No significant differences among the treatments were found for sensory qualities. The yogurt produced from 4% alginate-1.5% taro starch-encapsulated LAB, which was stored for 2 weeks, was found to be acceptable due to its right degree of sweetness and acidity and similarity with the control treatment. In this study, taro starch was proven to be a potential wall material that can be used with alginate to encapsulate yogurt starter cultures and can retain or increase the lactic acid bacteria cell viability without affecting the activity of the culture.

The Development of Indigenous Strain on Fermented Milk Product: A New Strain Combination

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ABSTRACT

The health benefits of food with live microbes (probiotics) on human are being widely promoted by health professionals. The purpose of this study was to develop a new strain on fermented milk product using indigenous strain. Rahayu, *et al.* (2013) successfully isolate five strains from Indonesian fermented foods, namely Dad-13, T-3, Mut-7, and Mut-13 which were identified as *Lactobacillus plantarum* and *Lactobacillus paracasei* SNP-2 which originated from infant intestine. The strains were indicated as probiotic candidates based on their resistance toward bile salt, simulated gastric juice, and antagonism ability. In this study, one of the probiotic strain (*L. plantarum* MUT7) together with *Streptococcus thermophillus* DAD-11 were used to produce fermented milk. The acceptability of fermented milk was tested using sensory test. The test was carried out to differentiate M7D11 product from commercial fermented milk (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*). The result of the sensory evaluation showed that M7D11 was preferred than the other one (p= 0.000). The significant differences were in mouthfeel (p= 0.005), sweetness (p= 0.000), and acidity (p= 0.001). However, there were no significant differences in viscosity (p= 0.278) and lingering after taste (p= 0.499). Hence, it can be concluded that fermented milk from M7D11 was preferred than the commercial fermented milk and will be developed in industry.

Keywords: fermented milk, sensory evaluation, starter culture, *Lactobacillus plantarum* Mut-7

Combination between Salt Concentration and Fermentation Temperature on Probiotic Capability and Antimicrobial Activity of Lactic Acid Bacteria from Isolation of Yellow Betung Bamboo Shoot Pickle

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ABSTRACT

Yellow betung bamboo shoot is the most popular fermented food for traditional snack *Lumpia* in Semarang. Mostly people in Indonesia know about yellow betung bamboo shoot and using it for their cooking. Bamboo shoot is known for its health benefits which sometimes used for medicinal purpose. Bamboo shoot can be processed as a pickle through fermentation in salt solution. The aim of this research is to determine probiotic capability and antimicrobial activity of lactic acid bacteria (LAB) from yellow betung bamboo shoot. All isolates were identified and confirmed as LAB. In 2.5% of salt solution at 15°C was found 20 isolates identified as *Lactobacillus* and 1 isolate as *Streptococcus*, 5.0% of salt solution at 15°C was found 18 isolates identified as *Lactobacillus* and 4 isolates as *Streptococcus*. In 2.5% of salt solution at 30°C (22 isolates) and 5.0% of salt solution at 30°C (27 isolates) were identified as *Lactobacillus*. All isolates from different salt concentration and temperature were recognized have probiotic capability and antimicrobial activity. The isolates need to optimize its environment to induce bacteriocin production and characterization.

Keywords: lactic acid bacteria, yellow bamboo shoot pickles, probiotic, antimicrobial

In Vitro Detection of Bacteriocin Inhibitory Activity of *Lactobacillus sp.* Isolated from *Betung* Bamboo Shoot (*Dendrocalamus asper*) Pickles under Different Fermentation Conditions and Medium Compositions

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ABSTRACT

Bamboo shoot is one of the edible materials which abundantly available in Indonesia. Since processed bamboo shoot has relatively short shelf life, other treatment which can extend the shelf life of bamboo shoot is needed. Pickling bamboo shoot is one of methods that can extend its shelf life. To date, pickled bamboo shoot is likely to have potency to be lactic acid bacteria (LAB) source especially bacteriocin-producing LAB. Bacteriocin of LAB is found to have antagonism activity against pathogenic bacteria which can cause spoilage in food product, food-borne diseases, and even death. However, the study to determine the effect of fermentation conditions and medium compositions on bacteriocin production from pickled bamboo shoots is still limited. The aim of this study is to determine the bacteriocin inhibitory activity of *Lactobacillus sp.* isolated from fermented *Betung* bamboo shoot under different fermentation conditions and medium compositions. *Lactobacillus sp.* with certain fermentation condition: 15°C in 2.5% of salt solution (A), 15°C in 5% of salt solution (B), 30°C in 2.5% of salt solution (C), and 30°C in 5% of salt solution (D) will be used. The effect of fermentation conditions on bacteriocin inhibitory activity will be analyzed by its antibacterial activity against three pathogenic bacteria: *Escherichia coli, Staphylococcus aureus,* and *Listeria monocytogenes* using agar-well diffusion. The effect of medium composition (carbon and nitrogen source) on bacteriocin production are also evaluated. Inhibitory activity by bacteriocin were observed by

measuring the clear zone and calculating activity unit (AU in mm^2/mL) of bacteriocin.

Keywords: bacteriocins, inhibitory activity, *Lactobacillus sp*., pathogen, pickled bamboo shoots

Adhesiveness and Microstructure of Rehydrated Avocado-Fortified-Yogurt

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ABSTRACT

This research aimed to analyze the adhesiveness and the profile of microstructure of avocado-fortified-dried yogurt. Yogurt was made from pasteurized bovine fresh milk at 72°C for 15 seconds, then inoculated with 4% (v/v) *Streptococcus thermophillu*s and *Lactobacillus bulgaricus*, and incubated at 38°C for 4 hours. The fortification of avocado 5% (v/v) was applied before pasteurization. Yogurt was dried in controlled cabinet dryer at 50°C to decrease the water content. Five grams of dried yogurt was rehydrated with 85 ml aquadest to produce 100 ml rehydrated yogurt. The adhesiveness data of rehydrated yogurt with avocado fruit extracts was $50.00 \pm 1.30 \times 10^6$ Nm while those without the addition of fruit extracts was $60.00 \pm 1.70 \times 10^6$ Nm. This data means that the addition of avocado did not have a considerable influence on yogurt adhesivity. However, based on microstructure observation, grain yogurt with fruit extracts of avocado was seen united among granules yogurt. While the yogurt without addition of fruit extracts had separate structure.

Keyword: rehydrated yogurt, adhesivity, microstructure, avocado

Biodegradation of Anthocyanin Using Beta Glucosidase from *Pediococcus pentosaceus* N11.16

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ABSTRACT

Anthocyanin glycoside is a major food compound which has important role in color formation, such as purple, blue, and dark red. From the previous research, we have isolated lactic acid bacteria from Indonesian fermented food. The isolate was identified as *Pediococcus pentosaceus* N11.16 and it can produce β-glycosidase. This enzyme is hydrolytic enzyme which can degrade anthocyanin glycoside. The aim of this research was to study the degradation of cyanidin-3-glucoside which broadly spread in the plant materials using β-glycosidase enzyme from *Pediococcus pentosaceus* N11.16. Enzymatic evaluation was carried out using para-nitrophenol as product indicator and para-nitrophenyl-β-D-glycopyranoside as a substrate. In this study, the enzyme was also tested using cyanidin-3-glucoside as a substrate.

Keywords: beta glucosidase, anthocyanin

A Novel Class Nutrient, MicroRNA, Content in Dairy Food Products

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ABSTRACT

MicroRNAs (miRNAs) is small (~21 nucleotides long) non-coding RNA that regulate almost 60% of human genes by post-transcriptional modification through translational inhibition or mRNA degradation. Recently, miRNAs are present in cow's milk and can be absorbed in the meaningful quantities amount in human. However, the presence of miRNAs in other dairy product is not investigated yet. Thus, the aim of this study was to investigate the presence of miRNA (miR-200c and miR-29b) in several dairy products: cheese, commercial yogurt, whip cream, half and half, and dip. Total RNA was isolated from dairy product using modified TriZol method followed by quantitative real time PCR to detect the presence of miR-200c and miR-29b in dairy product. miR-SPIKE was added to quantify the miR-200c and miR-29b. miR-200c and miR-29b were detected in dairy milk products. However, compared to skim milk, miR-200c was significantly low in all dairy milk products. Interestingly, miR-29b was significantly higher in greek yogurt and dip compared to skim milk. We conclude that processing of milk into dairy product will change a significant amount of miRNA level.

Keywords: microRNA, dairy food product, procesing

Isolation of Human Origin Methyl Mercury Resistant LAB from Sekotong, West Lombok, Indonesia

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ABSTRACT

Residences of Sekotong, West Lombok, had lived in mercury contaminated environment. This condition probably affects the condition of microflorae living inside residences' digestive tracts, i.e. occurrence of mercury-resistant Lactic Acid Bacteria (LAB). This study was aimed to isolate mercury-resistant LAB from faeces and breast milk of the residences of Sekotong. The procedures of collection had been accepted by the ethics committee of Faculty of Medicine, UGM twelve. Stools samples and 10 breast milk from 19 adult subject whom had lived at least for 5 years and didn't consume any antibiotic for minimal 2 months before samples collection were used in this study. The isolation was conducted by inoculation onto MRSA medium supplemented with 5 ppm methyl mercury. The positive isolates indicated by positive results in gram staining and catalase activity were selected for further examination. Out of these samples, 53 methyl mercury resistant LAB isolates were isolated. This present study implied that mercury contamination due to ASGM (Artisanal Small Gold Mining) activities in Sekotong had impacted the human health, especially the diversity and characteristics of digestive tract and breast milk microflorae.

Keywords: Lactic Acid Bacteria, Mercury Resistant LAB.

Effects of Temperature and Inoculum Concentration on Chemical and Microbiological Changes of Black Soybean Milk Yogurt

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ABSTRACT

Research objective was to study the effects of temperature and inoculum concentration on pH, titratable acidity, and the growth of lactic acid and acetic acid bacteria during fermentation of black soybean milk using *Caspian Sea Yogurt* as starter culture. Unpeeled black soybean milk added with 8% sucrose were inoculated with *Caspian Sea Yogurt.* The main microorganism involved in *Caspian Sea Yogurt* were *Lactococcus lactis* subsp. *cremoris* and *Acetobacter orientalis*. Fermentation was conducted for 18 hours at various temperature (24, 26, 28, 30, and 32^{o}C) and inoculum concentrations (1, 2, 3, 4, 5, and 10%). The optimum temperature for fermentation of black soybean milk by *Caspian Sea Yogurt* was 30°C. The fastest pH decrease and acid production were obtained at fermentation temperature of 30^{o}C and 5% inoculum. During black soybean milk fermentation at 30°C for 18 h, the pH decreased from 6.23 to 3.89, titratable acidity increased from 0.16% to 0.73%, and viable count of lactic acid and acetic acid bacteria reached 2.7x10^{8} and 5.1x10^{8} CFU/g respectively.

Keywords: black soybean milk yogurt, Caspian Sea Yogurt, fermentation, chemical and microbiological changes

Diversity of Lactic Acid Bacteria and Nutrition Content of "Yoguku" During Storage

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ABSTRACT

Yoguku is a yogurt produced by one of the famous factory in Malang. During the process, lactic acid bacteria is needed. The number and activity of lactic acid bacteria depend on nutrition content of raw materials and condition during storage. The objectives of this research were to observe the diversity and the number of lactic acid bacteria also nutrition content of the product during storage. After processed, *Yoguku* yogurt was stored at refrigerator (4°C to 10°C) for two months. Every two weeks, four bottle of samples were analyzed for the nutrition content (protein, fat, and lactic acid) and lactic acid bacteria number. Isolation of lactic acid bacteria was done using MRS agar and diversity was measured using diversity index and dominance index of Simpson. Identification of lactic acid bacteria was performed using API test kit 50CHL continued by 16S rDNA. Six isolates were obtained from *Yoguku* yogurt and the number of cells was not significantly different during storage. The highest diversity index 0.7 was observed at 0 week of storage, then after 8 weeks of storage, the diversity index decreased to 0.41. Identification of three isolates BYY1, BYY5, and BYY6 using API test kit 50CHL resulted in *Lactobacillus paracasei* ssp. *paracasei* 1 with similarity of 99%. However, isolate BYY2 was identified as *Lactobacillus brevis* with similarity of 65.4%. Identification using 16S rDNA resulted that isolate BYY2 was identified as *Lactobacillus plantarum* ssp. *plantarum* ZZU283 and isolate BYY5 as *Lactobacillus casei* MGB65-2 with similarity of 100%. The nutrition content of *Yoguku* yogurt for lactic acid and fat content after 6 to 8 weeks storage still appropriate according to national standard of Indonesia. However, the protein content during storage did not appropriate with the standard.

Keywords: diversity, identification, lactic acid bacteria, nutrition content

The Quality of Fermented Milk Produced Using Human-Origin Lactic Acid Bacteria as Starters

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ABSTRACT

The aim of this experiment was to evaluate the quality of fermented milk produced using human-origin lactic acid bacteria as starters. Fermentation was performed on pasteurized cows milk added with skim milk, constituting a total solid of 18%, using a separate single starter of *Lactobacillus casei* strain AP, *Lactobacillus casei* strain AG, and *Pediococcus acidilactici* strain BE. Parameter observed were pH and acidity, nutritional quality including protein, fat, and lactose content, product viscosity, and total lactic acid bacteria. The results showed that differences on starter cultures did not affect pH, acidity, fat, lactose, and protein content of the products. Differences on LAB starters affected the viscosity of the fermented products. The highest score of viscosity (4.035.66±109.69 cP) was found on the fermented product using *Lactobacillus casei* strain AP as starter, followed by products obtained using *Pediococcus acidilactici* strain BE (3.109,00±40.00), and *Lactobacillus casei* strain AG (3.052,33±15.27) as starters. Lactose, fat, and protein content, acidity and pH, as well as total lactic acid bacteria were not significantly different among fermented products. The average of total lactic acid bacteria in fermented milk product also not different. However, the number increased during fermentation from 6.98±1.00 $\log_{10}$ CFU/ml to 8.15±0.61 $\log_{10}$ CFU/ml. As conclusion, the use of three strains of human-origin lactic acid bacteria as starter for dairy fermentation affected physical quality but not nutritional and microbiological qualities of the products.

Keywords: fermented milk, human origin, lactic acid bacteria, starter culture, viscosity

Exopolysaccharide Producing Lactic Acid Bacteria Isolated from Palm Sap (*Arenga pinnata*)

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ABSTRACT

Palm sap is an important product produced in several towns in Tomohon, North Sulawesi. The fresh juice is commonly consumed as refreshments by the local people and the main material for producing palm-sugar (brown sugar), and is used for making local alcoholic drink named *saguer*, vinegar, and bioethanol. The natural occuring bacteria in palm wine mainly belongs from the group of lactic acid bacteria, fermented the sugar in palm sap to produce lactic acid. Rapid growth of LAB in fresh palm sap produce excess amount of lactic acid, which lower the pH of palm sap, resulted low quality of palm sugar. In addition, the presence of LAB make palm sap cloudy due to production of exopolysaccarides (EPS). Since the EPS is important in food industry, it is great interest to isolate and characterize EPS producing LAB from palm sap.

Lactic acid bacteria were isolated from palm sap obtained from different village in Tomohon such as Lahendong, Taratara, and Kayawu. Fresh palm sap samples were cultured on MRS broth and the LAB was isolated randomly from agar plates. Total of 61 isolates were obtained and screened for EPS production using *Sucrose Yeast Peptone Agar*, and 20 out of 61 isolates were selected as EPS producing LAB were identified by partial sequencing of the 16S rDNA. The results revealed that the EPS producing LAB closely related to *L. plantarum, L. buchneri, L. casei, L. brevis* and *Leu. Mesenteroides.* The *L. casei* AL.15 was selected for further studies for EPS production. The EPS was produced in 750 ml MRS culture broth after incubation for 48 hours at 37^{o}C. It was found that the *L. casei* AL.15

could produce 0.141 mg/L dry EPS. These results suggest that the *L. casei* AL.15 is potential for EPS production, but the resulted EPS requires further extensive studies.

Keywords: palm sap, lactic acid bacteria, exopolysaccharide

POSTER PRESENTERS

Effect Addition of Noni Fruit Extract (*Morinda citrifolia*) on The Growth of *Lactobacillus acidophilus* as Synbiotic to Inhibit Growth of *Salmonella typhi*

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ABSTRACT

Probiotics are microbes that normally live in the intestine to suppress the growth of pathogenic bacteria. Prebiotic selectively stimulating the growth of probiotic. Combination of probiotic and prebiotic known as synbiotic. Pectin is one of the compound that has the potential to act as a prebiotic and can be combined with *Lactobacillus acidophilus*. *Salmonella typhi* was the cause of typhoid fever. This study aimed to test the ability of noni fruit's extract in promoting the growth of *L. acidophilus* and to test the ability of synbiotic noni fruit's extract and *L. acidophilus* to inhibit the growth of *S. typhi*. This research used a quasi-experimental by two treatment groups. The first group tested the effect of noni fruit's extract to promote the growth of *L. acidophilus* as synbiotic to inhibit the growth of *S. typhi*, and the second group tested the effect of antimicrobial on the noni fruit's extract toward the growth of *S. typhi*. The concentrations of the extract were 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm, while the concentration of the suspension *L. acidophilus* and *S. typhi* was 1.5 x 10^6 CFU/ml. The results of the study showed that extracts of noni could promote the growth of *L. acidophilus* with P value of anova test P<0.05, while synbiotic *L. acidophilus* and noni fruit's extract could inhibit the growth of *S. typhi* with P value of regression test P<0.05 and IC_{50} 2.36 x 10^7 CFU/mL. The antimicrobe on noni fruit's extract does not have any effect on the growth of *S. typhi* with P value of regression test P>0.05. The conclusion of this research was noni fruit's extract could promote the

growth of *L. acidophilus* and could be combined as synbiotic which were capable to inhibite the growth of *S. typhi*.

Keywords: noni fruit extract, synbiotic, *S. typhi, L. acidophilus*

Recovery of Fermented Inulin Fiber by Lactic Acid Bacteria (LAB) from Inulin Hydrolysate Using Inulinase Enzymes of *Scopulariopsis* sp.-CBS_1 and *Deuteromycetes* sp.-CBS_4 as Cholesterol Binder

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ABSTRACT

Fermentation of Lactic Acid Bacteria (LAB) which are mixtures of *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus bulgaricus* and *Streptococcus thermophillus* on hydrolysate as a result of series of inulin hydrolysis using endophite fungi inulinase enzymes of *Scopulariopsis* sp.-CBS_1 (inulin hydrolysate of S) and *Deuteromycetes* sp.-CBS_4 (inulin hydrolysate of D) generate potential fermented inulin fiber as cholesterol binder. Fermentation process was conducted under concentrations of inulin hydrolysate 50% (w/v), *LAB* 15% (v/v) and skim milk 12.5% (w/v), room temperature and 40ºC for 0, 12, 24, 36, and 48 hours, respectively. Result of experimental work showed that based on Cholesterol Binding Capacity (CBC), optimization of fermentation process on inulin hydrolysate of S was achieved by combining treatment room temperature and 48 hours at CBC pH 2 of 18.281 (mg/g TDF) and inulin hydrolysate of D was achieved by combining 40ºC and 48 hours. At this optimal condition was resulted fermented inulin fiber from inulin hydrolysates of S and D with compositions of total acids of 0.918 and 0.792%, total sugar of 1199.2 and 1110.61 mg/mL, reducing sugar of 211.5 and 189 mg/mL, Total Plate Count (TPC) of 8.192 and 8.901 log CFU/mL, Soluble Dietary Fiber (SDF) of 0.665 and 2.151% (dry weight), Insoluble Dietary Fiber (IDF) of 10.645 and 8.037% (dry weight), total solids of 17.11 and 23.67% with CBC pH 2 of 18.281 and 24.281 mg/g and CBC pH 7 of 12.984 and 16.656 mg/g, respectively. Inulin hydrolysate of D fermented by LAB had better functional property as cholesterol binder than that inulin hydrolysate of S fermented by LAB. This is due to cholesterol binder and cholesterol derivatives as a result of degradation of

LAB on digestive system (stomach) when compared to higher colon under optimal process condition.

Keywords: fermented inulin fiber, CBC, LAB, *Scopulariopsis* sp.-CBS_1, *Deutrymecetes*-CBS_4

Drying Process of Fermented Inulin Fiber Concentrate by *Bifidobacterium bifidum* as A Result of Concentration Process Using Ultrafiltration System as Dietary Fiber Source for Cholesterol Binder Instant Drink

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ABSTRACT

Fermented inulin fiber as cholesterol binder has been performed by fermenting *Bifidobacterium bifidum* on hydrolysate as a result of fungi inulinase enzyme hydrolysis of *Scopulariopsis* sp-CBS$_1$. Their applications on preparation of drink was conducted through a series of concentrations process using Stirred Ultrafiltration Cell (SUFC) mode at rotation speed of 400 rpm and pressure of 40 psia for 45 minutes, drying process using vacuum dryer at 50ºC and 1 atm for 0, 8, 16, 24, 32, 40, and 48 hours. As comparison, drying process was carried out on fermented inulin fiber without ultrafiltration (UF) process. Based on optimization of Total Dietary Fiber (TDF), the best time of drying process was achieved for 40 hours. A longer time of drying process would increase TDF and total solids, decreased total acids, and fluctuated dissolved protein as well as Cholesterol Binding Capacity (CBC). Compositions of fermented inulin fiber powder resulted by drying process based on concentration method both using UF and without UF at optimum condition were total solids of 93.67 and 92.31%, TDF of 69.28 and 59.07% (dry weight), total acids of 7.5 and 7.03%, dissolved protein of 4.65 and 3.05 mg/mL,and CBC pH 2 16.8 and 15.71 mg/g, respectively concentration process using SUFC mode gave distribution of particles with better smoothness level than that without SUFC mode.

Keywords: fermented inulin fiber, ultrafiltration, drying, Cholesterol Binding Capacity (CBC), *Bifidobacterium bifidum*

Protective Effect of Milk Fermented by *Lactobacillus acidophilus* Against AFB_1 Exposed-Human Hepatoma Cells

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ABSTRACT

Aflatoxin B_1 (AFB_1), a group of mycotoxin, is the common contaminant found in foodstuff such as peanut and corn. It has been classified as 1A type of human carcinogen by International Agency for Research on Cancer. Aflatoxin-binding activity by *L. acidophilus* has been showed both *in vitro* and *in vivo*. Based on its evidence, we have investigated on the possible protective effects of milk fermented by *L. acidophilus* (MFLA) on the genotoxic effects of AFB_1. Human hepatoma HepG2 cells were exposed to AFB_1 and MFLA cocurrently. Therefore, we aimed to investigate cytotoxicity, oxidative DNA damage through 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation and its repair through human *OGG1* geneexpression in AFB1 treated-HepG2 cells. As analyzed by the MTT assay, MFLA reduced the cytotoxicity of AFB_1, when HepG2 cells were exposed to it before AFB1. LC-MS/MS analysis of 8-OHdG was carried out from treated and non-treated HepG2, whereas its repair was investigated using real-time *reverse transcription-PCR*. The 8-OHdG and *OGG1* expression were not significantly different for both before and after treated with MFLA compared to the control. However, both the formation and its gene expression were significantly increase after treated with non fermented milk. As a conclusion, MFLA was able to protect HepG2 cells from AFB_1-induced genotoxicity.

Keywords: *Lactobacillus acidophilus*, genotoxicity, hepatoma, cancer, MFLA

Prebiotics Activity of Laminaran

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ABSTRACT

The objectives of this study were to evaluate the prebiotic activity based on the change in cell biomass after 24 h growth of the probiotic strain in the presence of Laminaran Acid extract (LAE) and Laminaran Modified Extract (LME), inulin, or glucose relative to the change in cell biomass of *Escherichia coli* FNCC 0091 grown under the same condition. Prebiotic activity was observed on *L. plantarum* FNCC 0051 and *Bifidobacterium longum* FNCC 1081. The results showed that the growth of cell number of *L.* plantarum was high in both LAE and LME substrates (0.58 and 2.03 log cycle), whereas *B. longum* grow lower. High prebiotic activity was obtained on *L. plantarum* and *B. longum* grown on LME were positive (0.26 and 0.96 log cycle), whereas the lowest score was *L. plantarum* and *B. longum* grown on LAE were negative (-0.35 and -0.31 log cycle). High prebiotic activity was obtained on inulin (4,08 and 4,78 log cycle). It can be concluded that Laminaran Modified Extract (LME) has potency as prebiotic source, but it was lower than inulin.

Keywords: Laminaran Acid Extract, Laminaran Modified Extract, prebiotic activity score

The Characteristics of Yogurt with Different Commercial Starter Cultures

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ABSTRACT

Preparation of yogurt starter has been found to be the main concern in its application handled by villagers especially in order to establish yogurt home industry, in accordance with issue that yogurt home industry potential to improve the government's programme for food security and to increase the villager's source of income. The serious obstacle for improving the best starter resulted of isolations from commercial culture was it should meet the microbial standard parameter consistently so the starter subculture from commercial yogurt products is important. The objectives of this research was to determine the changes of total LAB, titratable acidity, pH, consumer preference for aroma and taste of yogurt. The four culture starters from commercial yogurt products were used. Starter 1, 2, 3 (S1, S2, S3: *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 from different yogurt products), and starter 4 (S4: *Lactobacillus bulgaricus* and *Streptococcus thermophillus*). Randomized Block Design was used in this research, with starter as a treatment and milk as a block, and repeated by five times. The results showed that total LAB in yogurt between starter S1 and S2, as well as between S2 and S3 were not significantly different ($P>0,05$), but in contrast, total LAB differences between starter S1 and S3 was significantly different. Titratable acidity and pH were not significantly different among yogurt product using starter S1, S2, S3, S4 ($P<0,05$). The consumer preference for yogurt aroma were not significantly different among all yogurt, and yogurt taste were not significantly different ($P>0,05$) for yogurts using starter S2, S3, S4, but yogurt with starter S1 were significantly different ($P<0,05$). All this results were found in accordance with the CODEX STAND 243-2003 for fermented milk standard.

Keywords: yogurt, commercial starter culture

Safety Assesment of Probiotic Candidate: The Effect of High Doses of *Lactobacillus plantarum* Dad13 to Rats Performance (Using Sprague Dawley Rats)

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ABSTRACT

Lactic acid bacteria (LAB) has been used in fermented food since many years ago. LAB as probiotic product known to have a health-promoting effect if consumed adequately. Because of the increased interest of LAB and probiotic product, several probiotic candidates have been isolated and identified from various sources. *Lactobacillus plantarum* Dad13 is a probiotic candidate isolated from *Dadih* (West Sumatra's traditional fermented food). Furthermore, *L. plantarum* Dad 13 has been examined its potential as a starter culture in industrial scale fermented milk production. The aim of this study was to evaluate the safety of *L. plantarum* Dad 13 in *Sprague Dawley* rats model for human consumption. Twenty *Sprague Dawley* rats were divided equally into five different groups. Initial control group without any treatment were sacrificed after adaptation phase. Treatment group were treated orally with high doses of *L. plantarum* Dad13 suspension (10^{11} CFU/ml per day) and the control group with control 1 ml per day for 14 and 28 days. Feed intake and body weight were evaluated as a general health status. Measurement of organ-weight, white blood cell, malonaldyaldehid concentration (MDA), Glutamic Oxalacetic Transaminase (GOT) activity, intestinal morphology, and organ microbiological analysis were used as safety parameters. Result showed that high doses of *L. plantarum* Dad 13 had no adverse effect on general health status, organ weight, white blood cell concentration, MDA concentration, GOT activity, and intestinal morphology. Those results suggested that *Lactobacillus plantarum* Dad 13 as probiotic candidate are likely safe for human consumption.

Keywords: safety assesment, probiotic, *Lactobacillus plantarum* Dad 13

Potential Use of Stirred Microfiltration Cell (SMFC) Mode in Separating Fermented Inulin Fiber by *Lactobacillus acidophilus* for Cholesterol Binder

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ABSTRACT

Process condition on Stirred Microfiltration Cell (SMFC) is a reference used to know process condition in larger process volume in module scale on separation process of fermented inulin fiber by *Lactobacillus acidophillus*. This biomass is rich source of dietary fiber used as Cholesterol Binding Capacity (CBC) relating with condition of digestive system (colon and stomach). Separation process of fermented inulin fiber by *Lactobacillus acidophillus* was performed using MF membrane (0.45 µm in pore size) fitted in SMFC under room temperature and fixed rotation speed and pressure (100 rpm, 40 psia) for 0, 30, 60, 90, and 120 minutes. The result of experimental activity showed that long time of separation process using SMFC retained and increased total solids, Total Dietary Fiber (TDF), total acids, dissolved protein, and CBC but decreased total sugars in retentate. SMFC mode passed and decreased total sugars, dissolved protein, and CBC but increased total solids, TDF, total acids in permeate, as well. Based on the optimum CBC, the best time of separation process was achieved in 120 minutes. In this condition generated fermented inulin fiber concentrate with concentrations of total sugar 105.21 mg/mL, total solids 2.11%, TDF 23.36%, total acids 6.66% (dry weight), dissolved protein 4.05 mg/mL, and CBC 13.781 mg/g. MF membrane was able to increase CBC 23.4% when compared without separation process under optimum process condition.

Keywords : Stirred Microfiltration Cell (SMFC), inulin fiber, Cholesterol Binding Capacity (CBC), retentate, permeate

Characteristic of Fermented Bayam (*Amaranthus* sp.) Polyphenol by Kambucha Culture as Antioxidant Compound as A Result of Stirred Microfiltration Cell (SMFC)

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ABSTRACT

Fermentation process of spinach (*Amaranthus* sp.) vegetable by kambucha culture as an attempt to get poliphenol as antioxidant compound had been done. Fermentation process was conducted on filtrate as a result of extracting one part of fermented spinach and four part of water under concentrations of 15% of kombucha culture (v/v) and 10% of sucrose (w/v), and at room temperature for 0, 7, and 15 days in closed container. Purification process of fermented spinach extract was microfiltered using Stirred Microfiltration Cell (SMFC) with fixed rotation speed of 400 rpm, room temperature, and pressure of 40 psia. Identification of polyphenol compound was carried out through LCMS using column C-18 (15 mm x 1 mm) with methanol eluent in injection volume of 2 µl and speed of 0.05 mL/minute. The result of experimental activity showed that long time of fermentation process increased concentrations of total acids, total polyphenol, and total solids in fermented spinach extract. Identification on molecular weight (MW) of polyphenol compound indicated polyphenol recovery with lower MW relating with long time of fermentation process. Spinach extract fermented for 0, 7, and 15 days displayed by dominant monomer were subsequent at T 3.0, T 2.7, and T 2.5, and T 2.5, T 2.9, and T 3.7. This matter demonstrated that long time of fermentation process increased functional property of poliphenol as antioxidant compounds.

Keywords: Stirred Microfiltration Cell (SMFC), spinach (*Amaranthus* sp.), kambucha, poliphenol, antioxidant

Characteristics of Sweet Corn (*Zea mays L. saccharata*) and Purple Sweet Potato (*Ipomea batatas*) Frozen Yogurt Added with Stabilizer

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ABSTRACT

Frozen yogurt (froyo) is a popular and fast growing functional food. Thus, it becomes one of the highly consumed desserts in worldwide. Recently, froyo can be made using alternative materials such as sweet corn (*Zea mays L. saccharata*) and purple sweet potato (*Ipomoea batatas*). Both of them have a good role as a medium for the growth of *Lactobacillus acidophilus* and *Bifidobacterium sp*. The aims of the study were to evaluate the sensory and physical characteristics (melting rate, viscosity overrun) of froyo. The research used carboxymethil cellulose (CMC), gum arabic, and carrageenan as a stabilizer with varied concentrations of 0.3% and 0.5%. All the samples were analyzed with hedonic test for sensory evaluation and analyzed for melting rate, viscosity, and overrun. The best formula then analyzed for total soluble solids, antioxidant activity, lactic acid level, and total lactic acid bacteria. The results showed that the melting rate of froyo ranged from 0.256 to 0.329 g/min, viscosity ranged from 159.60 to 596.07 cP, and overrun ranged from 4.312 to 23.273 %. The best formula based on sensory and physical characteristics was froyo with 0.5 % carrageenan. The sensory evaluation exhibited almost panelist like the colour, taste, mouthfeel, viscocity, and overall of the froyo. It was 0.330 g/min in melting rate, 596.07 cP in viscosity, 4.312% in overrun, 65.849% in total soluble solids, 85.305% in antioxidant activity, 0.34% in lactic acid level, and 9.94 $\log_{10}$CFU/ml in total lactic acid bacteria.

Keywords: frozen yogurt (froyo), carboxymethil cellulose (CMC), gum arabic, carrageenan

Potential of Yogurt as Angiotensin Converting Enzyme Inhibitor with Addition of *Ficus glomerata*

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ABSTRACT

Ficus glomerata Roxb. (Family: Moraceae) Syn. *F. racemosa* L. commonly known as Cluster fig in English. It is medium sized to large evergreen or occasionally deciduous tree and found all over India and Southeast Asia. *Ficus glomerata* Roxb. has been known to have flavonoids. Flavonoid compounds contained in plants and fruits are known to have the ability in inhibiting angiotensin converting enzyme. The purpose of this research was to study the ability of yogurt *Ficus glomerata* Roxb. as angiotensin converting enzyme inhibitors during storage at 4^0C. The results showed that the viability of lactic acid bacteria, total phenolic content, antioxidant activity, the value of o-phthalaldehyde (OPA), and Angiotensin Converting Enzyme inhibition on *Ficus glomerata*-yogurt showed significant differences ($p<0.05$) compared to plain yogurt at the end of the fermentation process up to seventh day of storage at 4^0C. The highest inhibition of angiotensin converting enzyme was in yogurt with fruit extract of *Ficus glomerata* 10% (69.11±0.50%) compared to yogurt with fruit extract of *Ficus glomerata* 5%(59.49±1.35 %), and plain yogurt (53.47±1.07%) on the seventh day storage and showed significant differences ($P<0.05$) for each treatment which parallel with the result of viability lactic acid bacteria and the value of o-phthalaldehyde (OPA). Viability of lactic acid bacteria in yogurt with fruit extract of *Ficus glomerata* 10% was 8.28±0.04 CFU/ml compared to yogurt with fruit extract of *Ficus glomerata* 5% was 8.14±0.06 CFU/ml and plain yogurt was 7.97±0.08 CFU/mL and shows significant differences. The value of o-phthalaldehyde (OPA) generated by yogurt with fruit extract of *Ficus*

glomerata 10% was 33.91±2.64 mg/g compared to yogurt with fruit extract of *Ficus glomerata* 5% was 29.80±2.32 mg/g and plain yogurt was 20.77±1.43 mg/g that showed significant differences ($P<0.05$) on the seventh day of storage at 4^0C.

Keywords: yogurt, angiotensin converting enzyme inhibitor, *Ficus glomerata*

The Potential of Lactic Acid Bacteria Isolated from *Dadih* as Antibacterial

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ABSTRACT

Dadih is a product of buffalo's milk obtained by fermentation. Fermentation of *Dadih* is made traditionally involve various microorganisms. Microorganisms which play a part in the fermentation are suspected from the inner part of bamboo pole surface, the surface of leaf cover, and the milk used. Milk fermentation products from lactic acid bacteria are known as healthy drink because it can prevent the activities and growth of various pathogenic bacteria.

This study aimed to isolate and characterize lactic acid bacteria from *Dadih* made of cow's milk. Cow's milk is used to replace buffalo's milk which is limited. The isolates were selected by their inhibitory ability on *Escherichia coli* and *Staphylococcus aureus.*

The main material used for the study was *Dadih* from cow's milk, MRS media for isolation and purification, MHA and NA for antibacterial activity test, Yeast-Peptone for acid production test. The analysis was performed by observation and morphological test of colony and cells, antibacterial activity test by well diffusion method, isolate physiological test by the influences of temperature, saline, pH media on isolates ability to grow, and isolate characteristic test to discover the genus and species of isolates.

The result showed that 8 isolates of Lactic Acid Bacteria were found, with 7 isolates have antibacterial activities on MHA media. One isolate coded as E7b2 potentially produce bacteriocins and 6 other isolates inhibit through the produced organic acids. On NA media, 7 isolates have antibacterial activities, 2 isolates coded as E7b2 and F4b1 potentially produce bacteriocins, and 5 other isolates inhibit through the produced organic acids. All isolates were Gram positive, catalase negative, and non-motile. Five isolates were homofermentative and 2 isolates were heterofermentative. The species from 3 isolates were *Lactobacillus*

acidophilus, 2 isolates were *Lactobacillus delbrueckii* subsp. *lactis*, 1 isolate was *Lactobacillus plantarum,* and 2 isolates were *Lactobacillus casei* subsp. *rhamnosus*.

Keywords: curd, lactic acid bacteria, antibacterial activity

Effect of Incubation Time and Sucrose Concentration on Probiotic Drink Characteristics from Whey a Cheese By-Product

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ABSTRACT

Whey is a cheese by-product that has potential to be developed into fermented drink because still have lactose content, protein and minerals. The aims of this study were to determine the effect of variations in incubation time and sucrose concentration on the probiotic drink characteristics made from whey. This study used Completely Randomized Design (CRD) with two factors, variation of incubation time (24 and 36 hours) and sucrose concentration (8%; 10%; 12%) on the probiotic whey drink. Probiotic starter cultures used were *Lactobacillus acidophillus* and *Bifidobacterium longum* IFO ATCC 13951 15 708 with a ratio of 1:1. Analysis was performed on viscosity, lactic acid levels, pH, antioxidant activity, total lactic acid bacteria and sensory characteristics. The results showed that the selected formula based on hedonic test is probiotic whey drink with 36-hour incubation time and 12% sucrose concentration. Probiotic whey drink has characteristics, namely viscosity 6.578 cP, 0.453% lactic acid levels, pH 4.36, the antioxidant activity of 19.409%, and total lactic acid bacteria 7.4 x 10^9 cells/ml.

Keywords: fermentation, incubation time, probiotic, sucrose concentration, whey

Profile of Sorghum Flour Modified Using *Lactobacillus brevis*

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ABSTRACT

Lactobacillus brevis is one of excellent species of lactic acid bacteria for modifying characteristics of flour that made the functional and physicochemical properties of flour is improved. This improvement is useful to increase the application of marginal flour for food products like sorghum flour. This research was conducted to identify the change of sorghum flour's profile that was modified using *Lactobacillus brevis*. The materials for this research were sorghum from KD 4 variety and *Lactobacillus brevis* isolate. Experimental design of the research was completely randomized using two factors and three replications. The first factor is microbial concentrations (0.5; 0.10; 0.15; 0.20%) and second factor is incubation times (4, 8, and 12 hours). The result showed that microbial concentrations and incubation times the cause changes of physicochemical properties such as starch content, amylose, bulk density and whiteness degree of flour. Mostly, rising of temperature causes the starch granule swelled and has high solubility. The solubility of sorghum starch was influenced by amylose content. Tannin content of the flour was decreased until 12 hours incubation. Water absorption of the flour has high range from 11.58 to 21.81%, whereas oil absorption of the flour has no significantly differences on several treatments. Microbial treatment causes the microstructure of sorghum starch granule changes into smaller pieces than the sorghum flour without microbial treatment.

Keywords: *Lactobacillus brevis*, modified, sorghum, profile

Isolation and Identification of Lactic Acid Bacteria from Fermentation Products and Its Potency as Antibacterial Agent

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ABSTRACT

Lactic acid bacteria can be found on many kind of Indonesian traditional fermented products. Viability of bacteria on foods are affected by many factors, including processing methods. Processing can change bacteria population on food. The aim of this research is to isolate lactic acid bacteria from various products with different processing methods prior to fermentation and to know its antibacterial activity.

In this research, lactic acid bacteria was isolated from various foods, such as *usar*, *tongcai*, *kincam*, salted radish, *teri asin*, *sedap malam*, salted fish, *tape singkong* and *tape ketan*, *gatot*, *tempe*, tofu *whey*, *ragi*, *ampas tahu*, and *kecutan tahu*. Isolation was done by pour plating method and purification streak plate method on MRS agar containing 1% $CaCO_3$ and 10 ppm Na-azide. The isolates were identified morphologically, biochemically, and physiologically. Antibacterial activity of the isolates were tested with agar well-diffusion assay on cell culture, acidic supernatant, and neutralized supernatant against *Staphylococcus aureus* and *Escherichia coli*.

The isolates were identified as *Lactobacillus* (5 isolates) from *kecutan tahu* and *ampas tahu*, *Pediococcus* (2 isolates) from *ragi tape* and salted fish, and *Streptococcus*/*Enterococcus* (9 isolates) from *tempe*, salted fish, and salted radish. Some of the isolates showed the antibacterial potency as they are able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* with inhibition diameter range from 9 to 15 mm. Inhibition of these pathogenic bacteria were caused by viable cell of lactic acid bacteria isolates and the acid produced during its growth.

Keywords: lactic acid bacteria, fermented products, antibacterial activity

Fermented Inulin Hydrolysate by *Bifidobacterium breve* as Cholesterol Binder in Functional Food Application

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ABSTRACT

Inulin hydrolysate is a result of inulin hydrolysis by inulinase enzyme of *Scopulariopsis* sp.-CBS1 fungi isolated from dahlia tuber skin in the formation of fructooligosaccharides (FOS) as dietary fiber. Inulin hydrolysate fermented by *Bifidobacterium* breve has a potential as cholesterol binder in digestive system due to its dietary fiber content. This study was conducted to evaluate the best cholesterol binding capacity by the variation of lactic acid bacteria (LAB) culture concentration of 10%, 20% and 30% (v/v), respectively. Fermentation process were conducted with inulin hydrolysate concentration of 25% (w/v), skim milk 7,5% (w/v), and various LAB culture concentration at 40 °C for 0, 12, 24, 36 and 48 hours. The results showed that the variation of LAB culture concentration affects the cholesterol binding ability in fermented inulin hydrolysate. The fermentation process with 10% LAB culture concentration at 40 °C for 48 hours resulted in the highest cholesterol binding capacity (CBC) of 13,69 mg/g at pH 7and 14,44 mg/g at pH 2 with the composition of total acid is 0,787%, soluble dietary fiber is 0,396%, insoluble dietary fiber is 5,47%, total solid is 14,476%, total sugar is 472,484 mg/mL, reducing sugar is 92 mg/mL, and total plate count (TPC) is 7,278 log CFU/mL, respectively.

Keywords: inulin hydrolysate, lactic acid fermentation, *Bifidobacterium breve*, dietary fiber, cholesterol binding capacity

Safety Assessment of Probiotic Candicate: Study of Bacteria Translocation on *Sprague Dawley* Rat's Organs and Blood Consuming *Lactobacillus plantarum* Dad 13 in High Dose

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ABSTRACT

Probiotic which is came from lactic acid bacteria (LAB), *Lactobacillus plantarum* Dad 13, derived from the isolation of West Sumatra's traditional fermented milk made of buffalo milk (*Dadih*) has been developed in Indonesia. This research examines the safety of consuming those bacteria in high dose. This research was conducted by observing the translocation possibility of *Lactobacillus plantarum* Dad 13 on *Sprague-Dawley* rats, which were treated with food contain high dose of *Lactobacillus plantarum* Dad 13 (10^{11} CFU/ml/rat/day) for 14 and 28 days. The result showed that there were 16 bacterias isolated from the rats' organs and blood grew in Lactobacillus Plantarum Selective Medium (LPSM). However, an identification using molecular analysis with rep-PRC method and BOX A1R primer showed that the bacteria found on those rats' organs and blood were not *Lactobacillus plantarum* Dad13. Based on that result, the rats consuming high dose of *Lactobacillus plantarum* Dad 13 (10^{11}CFU/ml/rat/day) for 28 days do not suffer any translocation whether in their liver, kidney, spleen, and blood. This result can be a supporting data to show the safety use of *Lactobacillus plantarum* Dad 13 as probiotic bacteria.

Keywords**:** probiotic**,** *Lactobacillus plantarum* Dad 13*,* rep-PCR*,* BOX A1R primer*.*

Screening of Uricase Producing Lactic Acid Bacteria and Evaluation of the Enzyme Stability in Gastrointestinal System

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ABSTRACT

Thirteen probiotic lactic acid strains were screening for uricase production and the result was evaluated to test the stability of this enzyme in gastrointestinal system. Screening uricase production by probiotics LAB strains was studied using PGY medium supplemented with uric acid as inducer. The result showed that all strains have uricase activities. It was evident that three strains namely *Lactobacillus* sp.OL5, *Lactobacillus plantarum* MUT7, and *Lactobacillus plantarum* DAD13 have the highest uricase activity. In addition, extracellular has the highest activity compared to intracellular and membrane bond in PGY medium. Those three strains were tested on gastrointestinal system. The result of this test showed that *Lactobacillus* sp. OL5, and *Lactobacillus plantarum* DAD13 produced intracellular uricase which remained active after gastric juice and duodenal test, yet the intracellular of *Lactobacillus plantarum* MUT7, extracellular and membrane bond enzymes of the three strains have no any activities.

Keywords: lactic acid bacteria, probiotic, stability, uricase

Effect of Heat Adaptation and pH Adjustment Pretreatments on Survival of *Lactobacillus paracasei* ssp. *Paracasei* SNP2 in Spray-drying

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ABSTRACT

The objectives of this research was to study the effect of heat adaptation pretreatment, pH adjustment, and its combination on the survival of stationary phase of *Lactobacillus paracasei* spp. *paracasei* SNP2 after lethal heat shock and after spray drying. *L. paracasei* SNP2 was grown in whey-sucrose medium at 37°C for 24 hours to get the mid-log phase and stationary phase. One percent (v/v) of 24 hours *L. paracasei* SNP2 starter culture was inoculated into test tubes containing whey-sucrose medium and incubated at 37° until mid-log phase (6 hours). Subsequently, the tubes were incubated at 37, 40, 42, 44, 46, 48, 50, and 55°C for 30 minutes, replaced immediately to ice water, and enumerated by dilution and plating method using MRS agar. Sub-lethal temperature ranges for adaptation and lethal temperature for lethal heat shock were determined. Heat adaptation temperature was selected from the range of sub-lethal temperature with the highest survival to the lethal shock. The cultures were pretreated with heat adaptation, pH adjustment with its combination, and enumerated after lethal heat shock and after spray-drying. The results showed that heat shock of *L. paracasei* SNP2 grown in whey-sucrose and MRS media at 55°C, 15 minutes, decreased the cell viability of 2.05 and 4.84 log cycles respectively. Heat adaptation at 44°C, 30 minutes, pH adjustment to 6.5 and its combination to *L. paracasei* SNP2 grown in whey-sucrose increased the survival against heat shock of 0.76; 0.55; and 0.54 log cycles respectively. The effect of these three pretreatments was more obvious in cell grown in MRS medium, indicated by survival increase to lethal shock

of 0,57; 1,05, and 2,40 log cycles respectively compared to untreated one. Heat adaptation and pH adjustment gave no significant effect of *L. paracasei* SNP2 survival in spray-drying.

Keywords: heat adaptation, pH adjustment, *Lactobacillus paracasei* SNP2, spray drying

Resistance of Two *Lactobacillus rhamnosus* in Gastrointestinal Tract of Rats Determined Using Species Specific Primers

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ABSTRACT

Resistance of probiotic in gastrointestinal tract is important criteria in development of functional probiotic. This work was carried out to elucidate the capabilities of two *Lactobacillus rhamnosus* isolated from different origins, infant feces (*L. rhamnosus* FBB42) and mare's milk (*L. rhamnosus* SKG34), to proliferate in the gastrointestinal tract of rats fed with high-fat containing diet. Four independent treatment groups consisted of eight rats were fed with high-fat containing diet (HF) including HF with Lbr SKG34: HF with Lbr FBB42; HF with Lbr SKG34 and Lbr FBB42 for 28 days. The rats were sacrificed and the presence of *L. rhamnosus* were determined using species specific primer for *L. rhamnosus* after the cecum content was enriched on MRS broth. The specific PCR revealed that *L. rhamnosus* could be detected in the cecum content of all probiotic treated rats, while it was absent in control treatments. This result indicated that *L. rhamnosus* SKG34 and *L. rhamnosus* FBB42 survived during the passage in the GI of rats and hence it could be promising to be developed as probiotic.

Keywords: probiotic, *lactobacillus,* specific primers

In Vitro Detection of Bacteriocin Inhibitory Activity of *Lactobacillus sp.* Isolated from *Betung* Bamboo Shoot (*Dendrocalamus asper*) Pickles Under Different Fermentation Conditions and Medium Compositions

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ABSTRACT

Bamboo shoot is one of the edible materials which abundantly available in Indonesia. Since processed bamboo shoot has relatively short shelf life, other treatment which can extend the shelf life of bamboo shoot is needed. Pickling bamboo shoot is one of methods that can extend its shelf life. To date, pickled bamboo shoot is likely to have potency to be lactic acid bacteria (LAB) source especially bacteriocin-producing LAB. Bacteriocin of LAB is found to have antagonism activity against pathogenic bacteria which can cause spoilage in food product, food-borne diseases, and even death. However, the study to determine the effect of fermentation conditions and medium compositions on bacteriocin production from pickled bamboo shoots is still limited. The aim of this study is to determine the bacteriocin inhibitory activity of *Lactobacillus sp.* isolated from fermented *Betung* bamboo shoot under different fermentation conditions and medium compositions. *Lactobacillus sp.* with certain fermentation condition: 15°C in 2.5% of salt solution (A), 15°C in 5% of salt solution (B), 30°C in 2.5% of salt solution (C), and 30°C in 5% of salt solution (D) will be used. The effect of fermentation conditions on bacteriocin inhibitory activity will be analyzed by its antibacterial activity against three pathogenic bacteria: *Escherichia coli, Staphylococcus aureus,* and *Listeria monocytogenes* using agar-well diffusion. The effect of medium composition (carbon and nitrogen source) on bacteriocin production are

also evaluated. Inhibitory activity by bacteriocin observed by measuring the clear zone and calculating activity unit (AU in mm^2/mL) of bacteriocin.

Keywords: bacteriocins, inhibitory activity, *Lactobacillus sp*., pathogen, pickled bamboo shoots

Effects of Sucrose Concentration on The Viability of *Lactobacillus plantarum* Dad 13 during Freezing, Freeze-Drying and Storage

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ABSTRACT

The aim of this study is to evaluate effects of sucrose as cryoprotectant on the viability of *Lactobacillus plantarum* Dad 13 during freezing, freeze drying, and storage. The inoculum was inoculated in whey-sucrose and incubated at 30°C for 24 h. The cells were harvested by centrifugation at 3000 rpm at 4°C for 20 minutes and re-suspended in 50 ml of sucrose cryoprotectant with the concentration 5, 10, 15, 20% (w/v). The suspensions were frozen at -44°C for 24 hours. The frozen cultures were then freeze-dried at -40°C for 72 hours. Total bacteria in the frozen and freeze-dried culture were enumerated using dilution and plating method in MRS and MRS-bile agar media. Before freezing the total viable cell was 11.41 log CFU. The results showed that addition of 5 to 20% sucrose cryoprotectant could maintain high cell viability after freezing with viable cell reduction in the ranges of 0.17 to 0.32 log cycles. There were some sub-lethal injury cells due to freezing that was shown by lower cell count in MRS with bile agar media. Sucrose also gave good protection during freeze drying process, with no significant different among sucrose concentrations. The viable cells were 10.73 log CFU, 11.04 log CFU, 11.20 log CFU, and 11.22 log CFU for 5%, 10%, 15% and 20% sucrose concentration respectively. The maximum decrease of viable cell after storage at frozen temperature for 4 weeks was 0.61 log cycle.

Keywords: *Lactobacillus plantarum* Dad 13, freezing, freeze-drying, sucrose

Screening for Anti-*Listeria* Activity of Lactic Acid Bacteria Isolated from Sea bream, *Sparus aurata*

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ABSTRACT

Lactic acid bacteria (LAB) are generally recognized as safe microorganisms, and bacteriocins produced by these bacteria have gained considerable interest as natural and safe preservatives in food and feed industries. Thus, this study aimed at screening of LAB strains which were able to produce a bacteriocin against *Lysteria monocytogenes*. A total of 52 LAB strains were isolated from Sea breams, *Sparus aurata*, and screened for their inhibitory activity against the food-borne pathogen using a microtiter-plate assay. The result indicated that cell free supernant (CFS) of one LAB strain exhibited significant inhibitory activity against the targeted pathogen. Based on their partial 16S rDNA sequences, the LAB strain showed high DNA similarity to *Enterococcus faecium* (NR113904; 99%).In addition, antimicrobial activity appeared to be quite stable between pH 2-10, and remained unchanged after treatment at temperature 30^{o}C-100^{o}C for 30 min, as well as at 121^{o}C for 15 min. However, anti-listeria activity of the CFS diminished after being treated with a proteolytic enzyme (proteinase K), indicating that it was a bacteriocin. These results may suggest that the bacteriocin produced by *E. faecium* could be potential as a natural food bio preservation for shell life extension.

Keywords: *E. faecium*, bacteriocin, *L. monocytogenes*

Lactic Acid Bacteria on Fermented Tigarun Flower (*Crateva nurvala* Buch. Ham) and Its Influence on Antibacterial and Antioxidant Activity

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ABSTRACT

Jaruk tigarun is a traditional fermented vegetable made from tigarun flower (*Crateva nurvala* Buch.Ham) fermented for 7 days. This study aimed to identify the lactic acid bacteria involved during fermentation and determine its influence on the antibacterial activity and antioxidant of jaruk. Isolation of LAB was carried out with MRS + CaCO3 media and identified physiologically, then continued with the identification kit API 50 CHL and the molecular identification using 16S rRNA sequences. Tigarun flower and jaruk were extracted using methanol solvent and the antibacterial and antioxidant activity were determined using well diffusion methods and DPPH (1,1-diphenyl,2- picrylhydrazyl) scavenging activity methods, respectively. From the identification using API 50 CHL, it was found that the group belongs to *Lactobacillus plantarum/L. pentosus* and *L. paraplantarum* based on molecular identification. Jaruk extract had a broad spectrum inhibition and showed some activity against the tested microorganism. *Salmonella* was the most resistant antibacterial followed by *S. aureus.* Antioxidant activity of jaruk was higher than the fresh one. The free radical scavenging activity of jaruk was 92.69±0.31 %.

Keywords: jaruk tigarun, fermented vegetable, lactic acid bacteria, antibacterial, antioxidant, DPPH

Resistance Test on Low pH and Deoxycholate Acid of Lactic Acid Bacteria Isolated from Goat Milk to Developed a Local Probiotic Candidate

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ABSTRACT

The aim of this study was to obtain lactic acid bacteria isolated from goat milk which is able to survive at low pH and sodium deoxycholic (NaDC), thus can be developed into a local probiotic candidate. The growth of lactic acid bacteria at low pH and deoxycholic acid were carried out using spectrophotometer (660 nm). A total of 100 lactic acid bacteria isolated from goat milk were able to survive when exposed to low pH 2.0, 3.0 and 4.0 and only 4 isolates were resistant to pH 2. The mechanism of lactic acid bacteria to survive at low pH (2 – 4) is remain unknown. This could possibly due to ATP-ase which is allow to maintain proton concentration in cytoplasm constantly. In addition, 62 isolates could survive from deoxycholic acid in a various range of concentration (0,2 mM, 0,4 mM and 0,6 mM) and only 5 isolates were able to convert cholic acid into deoxycholic acid. These results showed that lactic acid bacteria isolated from goat milk has a promising prospect to be a probiotic candidate.

Keywords: lactic acid bacteria, low pH, deoxycholic acid, cholic acid biotransformation

The Effect of Bacteriocin from Lactic Acid Bacteria against *Streptococccus agalactiae* Cause of Dairy Cattle Sub Clinic Mastitis

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ABSTRACT

The aim of this study was to know the antimicrobial activity of bacteriocin produced by *Lactic Acid Bacteria* (LAB) on *Streptococcus agalactiae* cause of dairy cattle sub clinic mastitis. The result showed that bacteriocin produced by LAB which was isolated from cattle intestine could inhibit the growth *Streptococcus agalactiae*. The failure of this bacteria growth was indicated by formation of clear zone surrounding the colonies on Brain Heart Infusion Agar plate. The heat stability of bacteriocin was evaluated by exposing to 80^0C for 30 minutes and 100^0C for 15 minutes, and it was also inactivated by enzyme trypsin. In conclusion, bacteriocin produced by Lactic Acid Bacteria was able to develop for therapeutic of dairy cattle sub clinic mastitis.

Keywords: bacteriocin, lactic acid bacteria, sub clinic mastitis

Viability of Lactic Acid Bacteria from Kombucha Tea Against Low pH and Bile Salt

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ABSTRACT

Kombucha tea is a functional drink fermented by various types of microbe. Kombucha tea is also a source of lactic acid bacteria that can maintain the balance of the microflora on the digestive tract which can improve the health of the human body. Lactic acid bacteria can act as a probiotic if it's able to survive in the human gastrointestinal tract, in order to reach the digestive tract, lactic acid bacteria has to be resistant to low pH in the stomach and bile salts. The purpose of this study was to determine the level of resistance of lactic acid bacteria in kombucha tea against low pH and bile salts. This study uses 19 isolates, each of these isolates was tested to the resistance of low pH 2.0 and 0.5% bile salts with incubation time of 4 hours. The results obtained indicate that 5 lactic acid bacteria isolates which resistant to low pH and bile salts were isolates MK31, MK42, RB210, RK41 and RS22. The isolates have huge potential to be developed as a probiotic candidate that can contribute greatly to the health of the digestive tract.

Keywords: kombucha tea, lactic acid bacteria, viability, low pH, bile salts

Isolation and Identification of Halophilic Lactic Acid Bacteria Produce Proteolytic Enzyme in Catfish Sauce (*Clarias species*) Processing

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ABSTRACT

This research was aimed to obtain halophilic LAB isolates that produce protease enzyme in the process of catfish sauce and to identify the genus of the bacteria. Halophilic LAB could be potent to be developed as a starter culture in fish sauce making. Catfish sauce was made by addition of 10% brine into 100 g of catfish. On the days of 3, 5, and 7d, samples were analyzed for the total acid bacteria producers. The colony counting was done using deMann Rogosa Sharp (MRS) medium using 1% CaCO3, 7% of NaCl, and Na Azida. For proteolytic enzyme, 2% of casein was added to the medium. Lactic acid bacteria were grown on the medium with 7% salt and proteolytic isolates with casein 2%, and then isolated and characterized by using a standard method, among others, including cell morphologic, gram dye, catalase test, gases production, growth on temperature, and pH. Bacteria that grow dominant were selected, then isolated in order to obtain pure 5 isolates ($553U_2$, $752U_2$, $754U_1$, $754U_2$, $757U_2$, and $764U_2$). According to the Bergey's manual of Bacteria Identification, the two isolates were identified as *P. pentosaceus.*

Keywords: halophilic LAB, isolation, identification, catfish sauce

Morphological and Physiological Identification of Lactic Acid Bacteria Isolated from Submerged Fermentation of Fermented Cassava (*Gatot*)

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ABSTRACT

Gatot is an Indonesian food product from spontaneous solid state fermentation of cassava. Before consuming, the *Gatot* must be soaked in the water for 3-5 days before drained and steamed for 30 minutes. During soaking process, occurred spontaneous fermentation and decrease of pH value that indicated the fermentation process by lactic acid bacteria (LAB). The aims of this research are to isolate and identified the lactic acid bacteria from submerged spontaneous fermentation (soaking process) of the *Gatot*. The LAB was cultivated on MRSA with $CaCO_3$ (3% w/v) to give clear zone around the LAB colonies. The LAB identification based on morphological properties and physiological (fermentation profile index) by using BBL Crystal kit. Morphological characteristics of isolated LAB was white round colonies, convex elevation, Gram positive, rod-shaped cell, no catalase enzyme activity, optimum growth at 37°C and cannot grew at 10°C and 50°C. Based on BBL Crystal identification showed that there were four strains of LAB i.e. *Lactobacillus manihotivorans*, *Bacillus licheniformis*, *Brevibacillus brevis*, and *Lactobacillus fermentum*. Based on traceability study, *L. Manihotivorans* was chosen to produce *Gatot* by controlled fermentation.

Keywords: lactic acid bacteria, submerged fermentation, *Gatot*, BBL Crystal

Survival, Heat Resistance and Antimicrobial Activity of *Lactobacillus* Strains Microencapsulated by Emulsion Method

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ABSTRACT

Microencapsulation is a technique that can be used to improve the viability of probiotic during food processing and through the intestinal tract. Two probiotic candidates (*Lb. plantarum* BSL and *Lb. plantarum* 2C12) were encapsulated using 3% sodium alginate and soybean oil (0.2% Tween 80). The objective of the study was to evaluate the effectivity of microencapsulation technique by emulsion method on the probiotic survival, heat resistance, bile salt tolerance (0.5%) and low pH (pH 2), and its antimicrobial activities. The results showed that both microencapsulated probiotics give good survival with high viability (11 log CFU g^{-1}). Heat resistance of the encapsulated strains at 50^0C was better than their free cells, although higher temperatures (60^0-70^0C) would lower the number of survivors. Heating at 50^0 - 70^0C caused injury to all probiotics cells either free or encapsulated. The survival of all encapsulated probiotics in bile salt and low pH, were also better than their free cells. All encapsulated *Lactobacillus* strains could inhibit the growth of *E. coli, Salmonella typhi* and *S. aureus* as well as their free cells, but none of them could inhibit the growth of *S. cereviseae* and *Aspergillus niger*. The results suggest that microencapsulation of probiotic by emulsion method is suitable for development of probiotic product.

Keywords: probiotic, microencapsulated, *Lb. plantarum*, emulsion method

Potency of *Lactobacillus Plantarum* B1765 as the Starter Culture of Soyghurt Fermentation

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ABSTRACT

This research observed the characteristics of soygurt product, which fermented by *L. plantarum* B1765 including pH, total acid, total of lactid acid bacteria (LAB), and total coliform. Fermentation were carried out for 24 h at 37°C. The β-glukosidase and antioxidant activities were also investigated. The pH and total acid were measured by AOAC 1995 method, the total LAB and total coliform were counted by Total Plate Count and the organoleptic test was tested using hedonic test. The β-glukosidase activity was measured at wavelength 402 nm, which showed the absorbance of p-nitrofenol liberated by digesting of p-nitrofenil-beta-D-glucopiranoside by the β–glukosidase activity. The antioxidant activity was determined by measured the non radical DDPH, which was formed by action of antioxidant in sample soyghurt to DDPH radical at 515 nm. Soygurt product has pH of 4.63, produce total acid of 3.47%, total LAB was 6.85x 10^{10} CFU/ml from initial inoculation of 10^6 CFU/ml, and could suppress the growth of coliform to 7.35 x 10^6CFU/ml. In the organoleptic test showed that the soyghurt sample was preference on the color, taste, and general preference level, but had low score in texture and aroma perception. The result of β-glucosidase enzyme activity (0.891 U/ml) was highest in 18 hours fermentation and the highest antioxidant activity was also formed in 18 hours fermentation with the IC_{50} was 12.29 mg/ml.

Keywords: soygurt, lactic acid bacteria, *L. plantarum* B1765, β-glukosidase, antioxidant

The Effect of Agitation in Mung Bean Cheese Production

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ABSTRACT

Mung bean (*Vigna radiata*) is a source of vegetable protein which can be used as raw material in the production of cheese. This study aims to determine the effect of agitation speed mixed cultures on the chemical characteristics of cottage cheese mung bean. Mung bean milk was made with different ratio of water and mung bean(1:6. 1:8 and 1:10). Mung bean milk was pasteurized and added with 10% mixed culture of *Lactobacillus bulgaricus* and *Streptococcus lactis*, then treated with agitation at 50 rpm and 100 rpm for 30 minutes. After incubation at 43°C for 48 hours, papain enzyme was added to the mung bean milk. Mung bean milk was incubated at 50°C for 36 hours and added with 5% salt. Based on the ANOVA calculation, total acid content, protein content, and fat content of the cheese were significantly difference.

Keywords: cottage cheese, agitation speed, mung bean

Milk Fermentation Using Freeze Dried *Lactobacillus plantarum* Dad 13

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ABSTRACT

Indigenous lactic acid bacteria potential as probiotic *Lactobacillus plantarum* Dad 13 has been used as inoculum in milk fermentation. The aims of this research are to determine the effect of incubation temperature and storage time of freeze dried *Lactobacillus plantarum* Dad 13 on cell growth, acid production, pH, and curd formation during milk fermentation. To determine the effect of incubation temperature, UHT milk with the addition of 2% skim milk (w/v) was fermented by adding 5 x 10^7 CFU/g freeze dried inoculum and incubated at 37°C and 42°C for 8 hours. To determine the effect of storage time, 5 x 10^7 CFU/g fresh and a week storage freeze dried *L. plantarum* Dad 13 were used as inoculum at 37°C. Milk fermentation was also carried out using liquid culture as control. During fermentation, the viable cells, pH, titratable acidity, and curd formation were monitored. The results showed that *L. plantarum* Dad 13 can grow faster at 37°C than 42°C. After 8 hours of fermentation at 37°C and 42°C, the total bacterial count were 1.9 x 10^8 CFU/ml and 4.35 x 10^7 CFU/ml with total acid 0.42% and 0.40%, respectively. There was no significant difference in milk fermentation using fresh and a week storage of freeze dried inoculum. The rates of bacteria growth, acid production, and reduction of pH were slower in milk fermentation using freeze-dried inoculum than that of with liquid inoculum. The curd was formed after 30 h fermentation.

Keywords: *Lactobacillus plantarum* Dad 13, milk fermentation, freeze drying, incubation temperature, storage time

Effect of Fermentation with *Lactobacillus casei* on the Physicochemical, Functional, Organoleptic and Microstructure Properties of Sorghum Flour

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ABSTRACT

Sorghum is one of the high carbohydrate food sources that the used of it in food processed still limited due to its anti-nutritional content and functional properties. To expand the use of sorghum in food products, it can be processed into intermediate product in the form of flour. Fermentation is one of the process that can reduce the anti-nutritional content and improve the functional properties of sorghum flour. The purpose of this study was to determine the effect of fermentation using *Lactobacillus casein* the physicochemical, functional, organoleptic, and microstructure properties of sorghum flour. The sorghum used in this study was local sorghum variety of KD-4 from Lamongan District, East Java. The experimental design used was completely randomized design with two factors: concentration of bacteria and fermentation time. The concentration of bacteria consist of four levels: 0.05%; 0.10%; 0.15%, and 0.20% of the volume of water, while the fermentation time were consist of three levels: 4 hours, 8 hours, and 12 hours. The number of bacteria colonies used were $9.65x10^{15}$. The results showed that the concentration of bacteria and fermentation time were generally affect on the physicochemical properties (proximate, whiteness, density, tannin, starch, amylose/amylopectin, and fiber), functional properties (viscosity, swelling power, solubility, gel consistency, water and oil absorption) and the microstructural change in starch granules of sorghum flour. However, the concentration of bacteria and fermentation time did not significantly affect the organoleptic properties of sorghum flour. Increase in the concentration of bacteria tend to decreased the tannin content, on the other hand, it increased the value of whiteness, dietary fiber, amylose, and oil absorption of sorghum flour. Increase in the fermentation time generally affect the increase value of density, and amylopectin of sorghum flour, but it

decreased the value of whiteness, water and oil absorption. While the starch, gel consistency, swelling power, and solubility tend to fluctuate.

Keywords: sorghum flour, *Lactobacillus casei*, physicochemical, functional properties

The Effectiveness of Synbiotic Fermented Milk Addition on Iron Supplementation in Iron Deficiency Children towards Gut Microbiota Balance

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ABSTRACT

The addition of synbiotic is expected to retain the balance of the intestinal microbiota because of iron supplementation. The aim of this study was to determine the addition of fermented milk on iron supplementation towards gut microbiota balance. This study was an experimental study on 58 iron deficient children (9-12 y.o), which have serum ferritin levels <15 mg/L. Subjects were randomly divided into 2 intervention groups, namely 1) given iron supplementation in syrup and 2) given iron supplementation in syrup and fermented milk using *L. plantarum* Dad 13 and FOS. Interventions carried out for 3 months. Hemoglobin levels, serum ferritin levels, body weight, height, and gut microbiota were measured at the beginning and end of the study. The results showed no difference after intervention on the total number of *Lactobacillus*, *Bifidobacteria*, and *E. coli*, but there was a significant increase on total *Enterobacteria* in group 1. There were no differences after intervention on total *Lactobacillus* but there was a significant increase in the total *E. coli, Bifidobacteria*, and *Enterobacteria* in group 2. There were significant increases after intervention on hemoglobin levels, ferritin serum levels, body weight, and height in both groups. There were no significant differences on total *Lactobacillus, Enterobacteria, E. coli*, body weight, height, hemoglobin levels, and serum ferritin levels after

intervention between 2 groups, but there was a significant difference on total *L. plantarum* Dad 13. Total number *L. plantarum* Dad 13 in groups 2 was higher than group 1. It was concluded that the given of fermented milk with synbiotic *L. plantarum* Dad 13-FOS can increase the number of beneficial bacteria but still can't decrease potentially pathogenic bacteria.

Keywords: hemoglobin, gut microbiota, iron deficiency, supplementation, fermented milk

Phenotypic and Molecular Identification of Folate-Producing Lactic Acid Bacteria

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ABSTRACT

Seventeen lactic acid bacteria isolates which were isolated from Indonesian traditional fermented milk (*Dadih*) has been known as folate producer between 12.43 to 29.27 µg/L. Folate represents an important vitamin B, which responsible in one-carbon transfer reaction required in many metabolic path-ways, especially purine and pyrimidine biosynthesis (DNA and RNA). This indicates the importance of folate in human metabolism. Identification analyzes of these strains were performed based on phenotypic and genetic characteristics. Phenotypic identification of isolates were carried out using API 50 CHL (BioMerieux, S.A., France), while molecular identification was performed based on PCR amplification and sequencing of 16SrRNA genes from lactobacilli. Based on phenotypic and molecular analysis, sixteen strains lactobacilli were identified as *Lactobacillus plantarum*, which were indicated by highly similarity level (phenotypic>91.9% and molecular identification>97%), and one strain was indicated by poorly similarity level (phenoypic 45.5 % and molecular identification 96%).

Keywords: lactic acid bacteria, folate, species identification

Degradation of Sesaminol Triglucoside in Sesame Milk Fermentation by β-glucosidase Producing *Lactobacillus plantarum* Dad 13

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ABSTRACT

Sesaminol triglucoside is a bioactive compound in sesame milk which has antioxidant activity. Sesaminol triglucoside exhibits higher antioxidant activity when it is hydrolyzed by β-glukosidase. The aims of this research are to study the increase of antioxidant activity and the decrease of sesaminol triglucoside concentration during sesame milk fermentation by *L. plantarum* Dad 13. Sesame milk was inoculated with *L. plantarum* Dad 13 and incubated at *37*^{o}C for 18 h. The viable cell, β-glucosidase activity, sesaminol triglucoside concentration, and antioxidant activity were monitored during fermentation. The crude extract of sesaminol glucoside lignan from defatted sesame seed was hydrolyzed using β-glucosidase. The antioxidant activity and the decrease of sesaminol triglucoside were analyzed. The results showed that *L. plantarum* Dad 13 grew well in sesame milk fermentation and produced β-glucosidase during fermentation. The antioxidant activity of sesame milk fermentation increased 2.34 times and sesaminol triglucoside concentartion decreased 56,4%. Hydrolysis of β-glucosidase on sesaminol glucoside lignan crude extract resulted in decrease of sesaminol triglucoside concentration and increase its antioxidant activity. It can be concluded that the increase of antioxidant activity was due to the degradation of sesaminol triglucoside by β-glucosidase that produced by *L. plantarum* Dad 13.

Keywords: enzyme β-glucosidase, sesaminol triglucoside, hydrolysis

The Effect of Skim Milk Concentration as Cryoprotectant on Viability of *Lactobacillus plantarum* Dad 13 during Freezing, Freeze-Drying and Storage

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ABSTRACT

The aim of this research is to study the effect of skim-milk concentrations on cell viability during freezing, freeze drying and storage in frozen temperature for 1 and 4 weeks. *Lactobacillus plantarum* Dad 13 was produced in whey 3%: sucrose 2% media, at 30°C for 24 h. After that, cultures were harvested by centrifugation and pellets were re-suspended with 50 ml skim-milk cryoprotectant at various concentrations (5%, 10%, 15%, 20% (w/v)), and as a control pellets were re-suspended in Phosphate Buffer Saline pH 7.0. After that, cellular suspension were frozen at -44 °C for 24 h. Freeze dying process was carried out at -40°C for 72 h. Freeze dried culture was stored at -44°C. The viable cells after freezing, freeze drying, and storage were enumerated using MRS and MRS with 0.15% bile media. After freezing, the viability of cells without cryoprotectant decreased 3.06 log cycles. The use of 10% skim-milk or above significantly increased cell viability. These were indicated by less than 1 log cycle reduction of viable cell. It was found that during freezing there were some sub-lethal injury cells. The use of 10 to 20% skim-milk cryoprotectant gave good protection during freeze-drying with viable cells reduction less than 0.5 log cycle. Storage of freeze-dried cells at frozen temperature for 4 weeks only reduced 0.38-0.5 log cycle of viable cells.

Keywords: freeze drying, cryoprotectant, skimmed milk, *Lactobacillus plantarum* Dad 13

Effect of Combination of Skim and Sucrose Cryoprotectant on Viability of Freeze-Dried *Lactobacillus plantarum* Dad 13

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ABSTRACT

The objectives of this study were to evaluate the effect of combination of skim milk and sucrose cryoprotectant on viability and sub lethal injury of *Lactobacillus plantarum* Dad 13. *Lactobacillus plantarum* Dad 13 was growth in 3% (v/v) whey and 2% (v/v) sucrose media at 30°C for 24 h, and then centrifuged at 3000 rpm, 4°C for 20 minutes. Cells were resuspended in skim milk-sucrose cryoprotectant with various ratios (1:9; 2:8; 4:6; 5:5; 6:4; 8:2). The suspensions were subsequently frozen at -40°C for 24 hours, and then subjected to freeze drying process at -40°C for 72 hours. Freeze-dried of *L. plantarum* Dad 13 was stored in -40°C for 4 weeks. Before freezing, the viable cells was 9.79 log CFU/ml. After freezing, the viable cells were in the ranges of 9.38-9.44 log CFU/ml in MRS agar with 0.20-0.26 log cycle sub lethal injury cells. Ratio of skim milk-sucrose cryoprotectant did not give any significant difference in cell viability. The viable cell of freeze-dried cultures were in the range of 9.94 to 10.12 log CFU/g. After 1 month storage at -40°C the viable cell of freezed-dried culture were 9.74 to 9.99 log CFU/g.

Keywords: skim-milk, sucrose, cryoprotectant, freeze drying, *Lactobacillus plantarum* Dad 13

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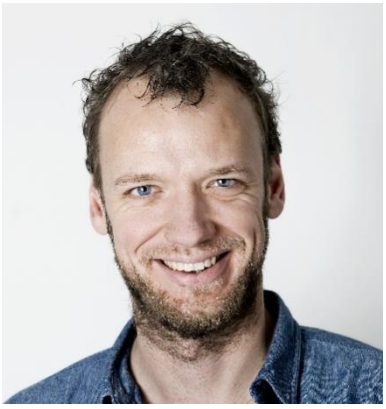

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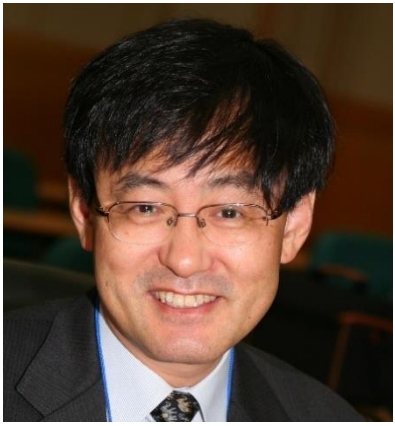

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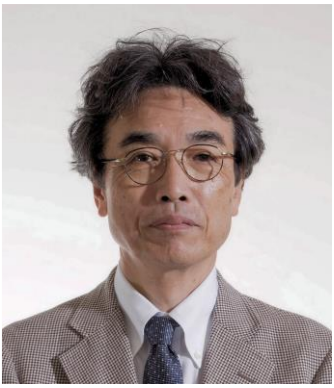

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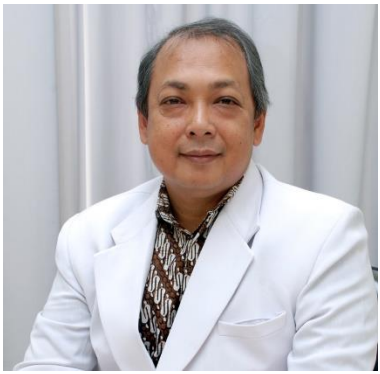

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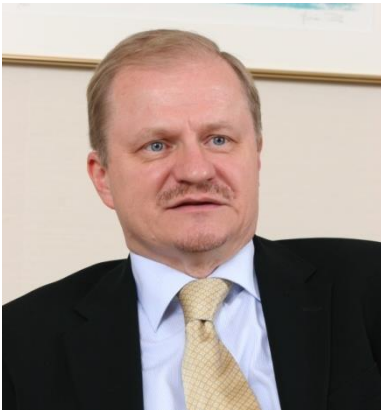

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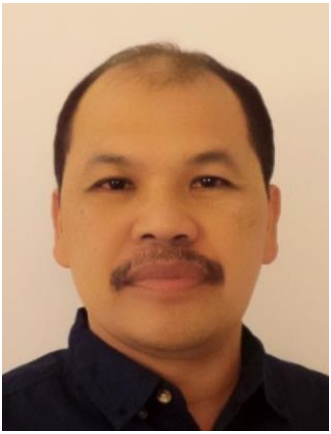

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