THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Biotechnology)

DEGREE

Biotechnology	Biotechnology
FIELD	**DEPARTMENT**

TITLE: Production of Spray-Dried Probiotic Lactic Acid Bacteria Grown in Cereal Extracts and Storage Stability

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THESIS

PRODUCTION OF SPRAY-DRIED PROBIOTIC LACTIC ACID BACTERIA GROWN IN CEREAL EXTRACTS AND STORAGE STABILITY

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Biotechnology)
Graduate School, Kasetsart University
2012

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Wanticha Lapsiri 2012: Production of Spray-Dried Probiotic Lactic Acid Bacteria Grown in Cereal Extracts and Storage Stability.
Doctor of Philosophy (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Associate Professor Penkhae Wanchaitanawong, Ph.D. 225 pages.

The objective of this study was to develop spray-dried cereal extract powders containing probiotic lactic acid bacteria. A total of 82 strains were isolated from fermented vegetables to screen for potential probiotics to be used in cereal extract fermentation. Thirteen strains of *Lactobacillus plantarum* were selected for their ability to produce high acid level and subsequently evaluated for their probiotic properties. Of 13 strains, *L. plantarum* TISTR 2075 was found to meet probiotic criteria including autoaggregation, coaggregation, cell surface hydrophobicity, antibiotic resistance, gastrointestinal tract tolerance, antimicrobial activity against *Escherichia coli* O157:H7 DMST 12743 and *Salmonella* Typhimurium ATCC 13311 and heat resistance considered as potential probiotic. Additionally, results showed that cereal flour (soybean, sesame and Job's Tears) exhibited the ability to protect *L. plantarum* during exposure to simulated gastrointestinal tract conditions. Soybean was found to improve the gastric tolerance of strains, while Job's Tears flour enhanced the small intestinal juice tolerance. Also, the viability of *L. plantarum* strains under simulated small intestinal juice after sequential of simulated gastric juice was quite stable with relatively high survival rate (>84%). Furthermore, the extract of these cereals supplemented with sesame could provide for good growth of *L. plantarum* TISTR 2075 with the viable cell count of >8.3 log CFU/mL and the significant increase in calcium solubility was also observed after 24 h fermentation. Spray drying of *L. plantarum* TISTR 2075 in maltodextrin with various protectants (protein, trehalose, fibersol, isomalt, ascorbic acid, palatinose and acacia gum) was also investigated. Protein and trehalose were considered as the most efficient protective media providing high survival rate of 97.4 and 93.3%, respectively. Also, probiotic properties including gastrointestinal tract tolerance and antimicrobial activity was not affected by spray drying process. Survival of the spray-dried strain was monitored over a period of 12-month storage at 4 and 25 °C. During storage at 4 °C, the strain retained its viability of 10^4–10^8 CFU/g over 12 months depending on protectant types. At an elevated storage temperature of 25 °C, a significant (P<0.05) loss of viability was observed in all protectants until reaching a total death at 4 months of storage. Moreover, the storage temperature and relative humidity were found to influence the viability of spray-dried *L. plantarum* TISTR 2075 and physical property of the powders. Increasing the relative humidity and temperatures resulted in higher loss of viability. A temperature dependent prediction model based on Arrhenius theory was developed to determine the viability of the spray-dried *L. plantarum* TISTR 2075 in different protectants for long-term storage.

Student's signature Thesis Advisor's signature ___ / ___ / ___

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ACKNOWLEDGEMENTS

I would like to express my deep and sincere gratitude to my advisor, Associate Professor Dr. Penkhae Wanchaitanawong, for her valuable advices, continuous support, encouragement, constructive criticism and kindness throughout my study which enable me to carry out this dissertation successfully. Besides, I would sincerely like to thank my thesis co-advisor: Associate Professor Dr. Sunee Nitisinprasert for her generosity, helpfulness, great suggestions, insightful comments and constant encouragement throughout my work. Additionally, I would like to gratefully and sincerely thank examination chairperson: Associate Professor Dr. Mangkorn Rodprapakorn and external examiner: Associate Professor Dr. Yuwapin Dandusitapunth for their insightful comments and suggestions.

I am particularly grateful to Professor Dr. Bhesh Bhandari, my co-supervisor at School of Agriculture and Food Sciences, The University of Queensland, Australia for allowing me the "once-in-a-life-time" opportunity to conduct my research in Australia. From his invaluable advices and constructive criticism, I acquired numerous knowledge produced the great improvement of my research. Without his patience and kindness it would be impossible to complete my study.

I would like to thank the Office of the Higher Education Commission, Ministry of Education, Thailand, under the program "Strategic Scholarships for Frontier Research Network for the Join Ph.D. Program, Thai Doctoral Degree" for the financial support throughout my Ph.D. study. I really appreciate this fabulous scholarship and supervision.

Furthermore, I would like to thank the Graduate school, Kasetsart University. Many thanks are also expressed to all colleagues in Department of Biotechnology, Faculty of Agro-Industry for their kindness, support and friendship.

Finally, I wish to express my greatly appreciation to my parents, grandmother and aunt for their love, understanding, patience, support, encouragement and everything throughout from the beginning.

Wanticha Lapsiri

April 2012

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LIST OF ABBREVIATIONS

AU/mL	=	Arbitrary units per milliliter
ca.	=	*circa* (Latin), in approximately
CFS	=	cell-free supernatant
CFU/mL	=	colony forming unit per milliliter
CFU/g	=	colony forming unit per gram
°C	=	degree Celcius
etc.	=	et cetera (and so on)
e.g.	=	*exempli gratia* (for example)
h	=	hour
g	=	gram
g	=	G-force
GIT	=	gastrointestinal tract
LAB	=	lactic acid bacteria
µL	=	microliter
M	=	molar
mg/L	=	milligram per liter
min	=	minute
mL	=	milliliter
mm	=	millimeter
MW	=	molecular weight
nm	=	nanometer
OD	=	optical density
ppm	=	parts per million
rDNA	=	Ribosomal DNA
sec	=	second
S.D.	=	Standard Deviation
T_g	=	Glass transition temperature
v/v	=	volume per volume
w/v	=	weight per volume

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PRODUCTION OF SPRAY-DRIED PROBIOTIC LACTIC ACID BACTERIA GROWN IN CEREAL EXTRACTS AND STORAGE STABILITY

INTRODUCTION

Food products combining probiotics have been gaining more interest and have been available in global markets which was worth US$16 billion in 2008 and the estimated target is a total of US$ 19.6 billion in 2013, representing a compound annual growth rate of 4.3% (Champagne *et al.*, 2005; Soccol *et al.*, 2010; Santivarangkna *et al.*, 2011). The beneficial effects of probiotics on human health has been claimed by several studies such as aid in reduction of lactose intolerance, resistance to enteric pathogen, prevention of cancer, cardiovascular disease, gastrointestinal disorders and stimulation of the host immune responses (Sanders, 2003; Iannitti and Palmieri, 2010; Ranadheera *et al.*, 2010). In recent years, consumers' demand for non-dairy based probiotic products has increased. The allergy to dairy products affects negatively some persons. Lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products. Therefore, such products might prove unsuitable for people with an intolerance of lactose or an allergy to milk proteins (Prado *et al*., 2008). Moreover, the increasing in the consumer vegetarianism throughout the developed countries is also a demand for the vegetarian probiotic products. At present, some non-dairy probiotic beverages are being commercialized and much of them are non-alcoholic beverages manufactured with fruits, vegetables or cereals as principal raw materials.

Cereals are considered to be the most important sources of protein, carbohydrates and fiber. Recently, technological advances have made possible to alter some structural characteristics of cereal matrices by modifying food components in a controlled way. This could make them ideal substrates for probiotic cultures since they already contain beneficial nutrients such as minerals, vitamins, dietary fibers and antioxidants (Granato *et al.*, 2010). Also, they can be used as sources of non-

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digestible carbohydrates acting as prebiotics to stimulate the growth of probiotics (Rivera-Espinoza and Gallardo-Navarro, 2010). Furthermore, they do not contain any dairy allergens that might prevent usage by certain segments of the population (Yoon *et al.*, 2004). Interestingly, the increase in calcium solubility related to lowered pH associated with production of organic acid was also observed during cereal fermentation (Tang *et al.*, 2007). The application of probiotic cultures in non-dairy products represents a great challenge for developing functional probiotic foods.

Drying techniques to obtain dehydrated probiotics in viable state have proven useful (Ananta, 2005; Sunny-Roberts and Knorr, 2011). In recent years, spray drying has been used for the preservation of probiotic bacteria due to its relatively inexpensive cost and easy availability of the process (Santivarangkna *et al.*, 2007; Bhandari, 2008; Bhandari *et al.*, 2008; Peighambardoust *et al.*, 2011). Viability and stability of probiotics have been a technological challenge because the probiotics bacteria are susceptible to high temperature during drying process. A prerequisite for probiotic products is that a sufficiently large number of viable probiotic bacteria survive (at least 10^6 CFU/g) in the final product at the time of consumption (Abe *et al.*, 2009b). Since the drying process may cause significant damage to the cell membranes, proteins and nucleic acids of bacteria, it is not easy to maintain living cells in foods for a long time under general conditions such as ambient temperature (Potts, 1994; Teixeira *et al.*, 1995a; Teixeira *et al.*, 1997; Santivarangkna *et al.*, 2008a; Silva *et al.*, 2011). The addition of protectants is practiced to protect cellular structures from thermal and dehydration damages (Potts, 1994; Chávez and Ledeboer, 2007; Strasser *et al.*, 2009; Silva *et al.*, 2011). Protectants such as sugars, skim milk, acacia gum or monosodium glutamate (MSG) have been also studied in order to improve the drying tolerance of strains (Castro *et al.*, 1995; Reddy *et al.*, 2009; Riveros *et al.*, 2009; Sunny-Roberts and Knorr, 2009; Golowczyc *et al.*, 2011b). Additionally, the synergistic effects may be acquired from the combination of different protectants (Santivarangkna *et al.*, 2007). Also, the stability of probiotic during storage is an important criterion. During storage, temperature and relative humidity are critical factors affecting retention of cell viability and physical property in terms of glass transition temperature (T_g) of spray-dried powder (Castro *et al.*,

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1995; Teixeira *et al.*, 1995b; Pehkonen *et al.*, 2008). However, the combined effects of temperature and relative humidity related to the glass transition temperature on the storage stability of microorganisms have not been investigated so far.

Therefore, this study is an attempt to develop cereal-based probiotic products which can be incorporated into fruit and vegetable juices as well as marketed as supplements in the form of spray-dried powders. Furthermore, the survival of probiotic in spray-dried powders with different protectants after spray drying and during storage was evaluated. Also, the correlation between the viability of probiotic and the physical property in term of the glass transition temperatures (T_g) of spray-dried powders during storage was determined in the present study.

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OBJECTIVES

1. To investigate probiotic properties of microorganisms isolated from fermented vegetables.

2. To evaluate the effect of indigestible cereal flour on viability of probiotic under gastrointestinal tract conditions.

3. To evaluate the effect of protectants on the stability of probiotic during spray drying process and storage.

4. To determine the glass transition temperature of various spray-dried powders kept under different conditions.

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LITERATURE REVIEW

1. Probiotics and their application in foods

Probiotic is generally used to name the bacteria associated with the beneficial effects for the humans and animals (Prado *et al.*, 2008). The term probiotic was technically defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). This means that the microorganisms must be alive and present in high numbers, generally more than 10^6–10^7 cells per daily ingested dose (Kosin and Rakshit, 2006; Karimi *et al.*, 2011).

The majority of probiotic microorganisms belongs to the genera *Lactobacillus* and *Bifidobacterium* which were used in food, nutrition and in pharmaceutical preparations (Mercenier *et al.*, 2003; Corcoran *et al.*, 2006; Lee and Salminen, 2009; Giraffa *et al.*, 2010; Iannitti and Palmieri, 2010; Khater *et al.*, 2010). *Lactobacilli* and *Bifidobacteria* are Gram-positive lactic acid-producing bacteria that constitute a major part of the normal intestinal microflora in humans and animals. Additionally, some strains of *Pediococcus*, *Bacillus* and yeast have also been found as suitable candidates (Giraffa *et al.*, 2010). Probiotics play a crucial role in the protection of the organism against harmful microorganisms and also strengthen the host's immune system. They are usually consumed after the antibiotic therapy which destroys the microbial flora present in the digestive tract (both the useful and the targeted harmful microbes). Probiotics can be found in dairy and non-dairy products (Prado *et al.*, 2008). As shown in Figure 1, probiotics are available and administrated in different forms mainly in a fermented state and pharmaceutical products (Holzapfel, 2006). Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance of the population of useful or beneficial microbes in the intestinal flora (Soccol *et al.*, 2010). The health benefits probiotics in human clinical trials is shown in Table 1.

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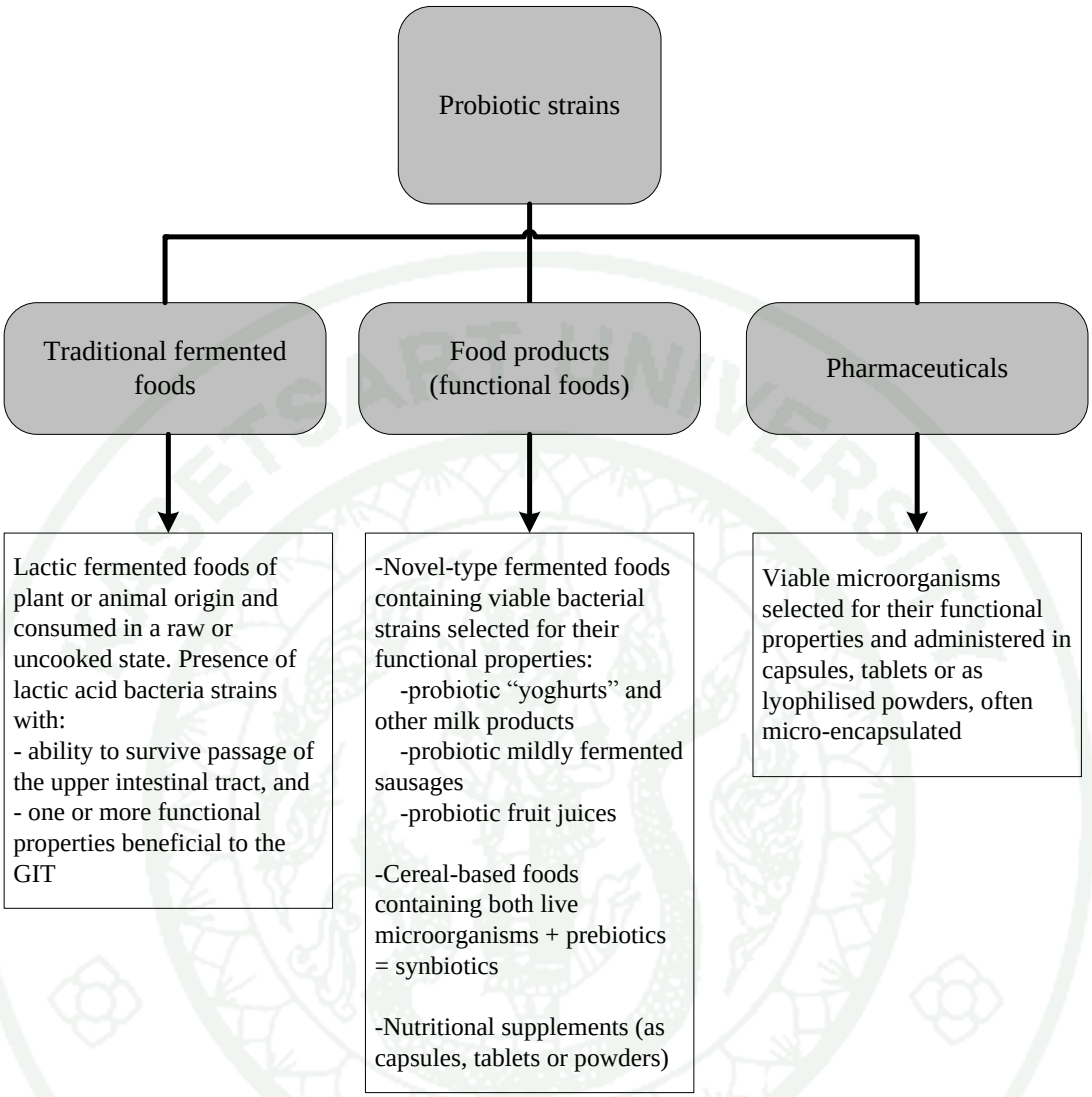

Figure 1 Administration of probiotics in different forms.

Source: Holzapfel (2006)

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Table 1 Example of health benefits probiotics in human clinical trials.

Microorganisms	Health benefits
Lactobacillus casei Shirota	Immune modulation
L. plantarum LP01 *Bifidobacterium breve* BR0	Relief of irritable bowel syndrome (IBS)
L. johnsonii La1	Reduced *Helicobacter pylori* colonization
L. acidophilus *B. bifidum*	Reduction of colonic infection of *Clostridium difficile*
L. salivarius VSL#3	Reduced symptoms associated with diarrhoea-predominant IBS
B. lactis Bb12	Allergy treatment
Saccharomyces bouladii	Reduction of antibiotic associated diarrhoea (AAD)
L. salivarius UCC118	Reduced inflammatory bowel disease (IBD)
L. rhamnosus GG	Shortening of diarrhoea

Source: Modified from Corcoran *et al.* (2006)

1.1 Criteria for selecting probiotic

The criteria currently used to select probiotics define the optimal quality control of probiotic strain in industrial practice. Furthermore, a certain technological and physical characteristic of probiotic strains are important (Vasiljevic and Shah, 2008). A general agreement exists with regard to key selection criteria listed in Table 2. A summary of conventional criteria that can be used for the selection of microbial strains as probiotics includes the following properties:

(i) Biosafety

One of the most important characteristics of a probiotic strain is that it must be non-pathogenic and generally recognized as safe (GRAS) status in order to safe use in foods (de Vrese and Schrezenmeir, 2008; Muller *et al.*, 2009). In theory,

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probiotics are responsible for 4 types of side effects including systemic infections, deleterious metabolic activities, excessive immune stimulation and gene transfer. In practice, *Lactobacilli* and *Bifidobacteria* are extremely rare causes of infections in humans. This lack of pathogenicity extends across all age groups and also to immunocompromised individuals (Soccol *et al.*, 2010). Three approaches are possible in the assessment of the probiotic safety including (i) studies on the intrinsic properties of the probiotic strain, (ii) studies on pharmacokinetics of the probiotic strain (survival, activity in the intestine, dose-response relationships, faecal and mucosal recovery and (iii) studies on interactions between the probiotic strain and the host (Saarela *et al.*, 2000; Soccol *et al.*, 2010).

Furthermore, the emergence of antibiotic resistant organisms is an ever increasing problem and a potentially serious threat to public health. In the past few decades, the problem escalated as the prevalence of antibiotic-resistant bacteria has been growing significant in healthcare sector. Also, multi-drug-resistant strains causing disease in humans have emerged. For this reason, the safety profile of a potential probiotic strain is of critical importance in the selection process. This testing should include the determination of strain resistance to a wide variety of common classes of antibiotics and subsequent confirmation of non-transmission of drug resistance genes or virulence plasmids. Ideally, probiotic bacteria should exhibit tolerance to antimicrobial substances used in clinical practice but it should not be able to transmit such resistance to other bacteria (Del Piano *et al.*, 2006).

(ii) The strain origin

Probiotics should preferentially originate from the target human or animal microflora. This choice is determined by the specific purpose of the application of the probiotics (e.g. location specificity or requirement for colonization). The strains should be properly isolated and identified before use (Morelli, 2000).

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Table 2 Key and desirable criteria for the selection of probiotics in commercial applications.

General	Properties
Safety criteria	Origin Pathogenicity and infectivity Virulence factors-toxicity, metabolic activity and intrinsic properties, i.e., antibiotic resistance
Technological criteria	Genetically stable strains Desired viability during processing and storage Good sensory properties Phage resistance Large-scale production
Functional criteria	Tolerance to gastric acid and juices Bile tolerance Adhesion to mucosal surface Validated and documented health effects
Desirable physiological criteria	Immunomodulation Antagonistic activity towards gastrointestinal pathogens, i.e., *Helicobacter pylori*, *Candida albicans* Cholesterol metabolism Lactose metabolism Antimutagenic and anticarcinogenic properties

Source: FAO/WHO (2002)

(iii) Ability to survival under gastrointestinal tract conditions

To reach the intestinal, strains must first pass through the stomach providing a powerful barrier to the entrance into gut (Dunne *et al.*, 2001; Huang and Adams, 2004). The secretion of gastric acid constitutes a primary defense mechanism

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against most ingested microorganisms. Probiotic strains must tolerate the acidic and protease-rich conditions of the stomach (Tuomola *et al.*, 2001). Moreover, the ability to survive the action of bile salts is generally included among the criteria used to select potentially probiotic strains (Saarela *et al.*, 2000). Bile resistance could differ among strains of one single species of enteric *Lactobacilli* (Morelli, 2000). Morelli (2000) and Del Piano *et al*. (2008) indicated that the resistance testing to bile is generally conducted with 0.3% pig or bovine bile (oxgall). Tolerance to gastric and bile acid as well as sufficient against digestive enzymes enable the survival during the passage through stomach and upper intestinal tract and have health-promoting effects in the gut (Lee and Salminen, 2009). Buffering capacity of ingested foods causes the lower decrease of pH in stomach. Therefore, resistance against gastric acid is less critical than tolerance of bacteria to bile acid and digestive enzymes in the small bowel (Del Piano *et al.*, 2006; de Vrese and Schrezenmeir, 2008).

(iv) Adhesion properties

Adhesion of probiotic strains to the intestinal surface and the subsequent colonization of the human GI-tract have been suggested as an important prerequisite for probiotic action (Lee and Salminen, 2009). Adherent strains of probiotic bacteria are likely to persist longer in the intestinal tract and have better possibilities of showing metabolic and immunomodulatory effects than non-adhering strains. Adhesion provides an interaction with the mucosal surface facilitating the contact with gut associated lymphoid tissue mediating local and systemic immune effects. Thus, only adherent probiotics have been thought to effectively induce immune effects and to stabilize the intestinal mucosal barrier (Morelli, 2000). Adhesion may also provide means of competitive exclusion of pathogenic bacteria from the intestinal epithelium (Saarela *et al.*, 2000).

(v) Antimicrobial activity

To have an impact on the colonic flora, it is important for probiotic strains to show antagonism against pathogenic bacteria via antimicrobial substance

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production or competitive exclusion (Saarela *et al.*, 2000). The primary antimicrobial effect exerted by probiotic is the production of lactic acid and reduction of pH. In addition, lactic acid bacteria produce various antimicrobial compounds which can be classified as low-molecular-mass (LMM) compounds such as hydrogen peroxide (H_2O_2), carbon dioxide (CO_2), diacetyl (2,3-butanedione), uncharacterized compounds and high-molecular-mass (HMM) compounds like bacteriocin (Tsai *et al.*, 2004; Ammor *et al.*, 2006; ; Lin *et al.*, 2006; De Vuyst and Leroy, 2007). All of which can antagonize the growth of some spoilage and pathogenic bacteria in foods.

Levels and types of organic acids produced during the fermentation process depend on strains of lactic acid bacteria, culture composition and growth conditions. The antimicrobial effect of organic acids lies in the reduction of pH as well as the undissociated form of the molecules. It has been proposed that the low external pH causes acidification of the cell cytoplasm, while the undissociated acid being lipophilic can diffuse passively across the membrane. The undissociated acid acts by collapsing the electrochemical proton gradient or by altering the cell membrane permeability which results in disruption of substrate transport systems. Lactic acid is the major organic acid of lactic acid bacteria fermentation which is in equilibrium with its undissociated and dissociated forms and the extent of the dissociation depends on pH (Ammor *et al.*, 2006).

H_2O_2 is produced by lactic acid bacteria in the presence of oxygen as a result of the action of flavoprotein oxidases or nicotinamide adenine dinucleotide (NADH) peroxidase. The antimicrobial effect of H_2O_2 may result from the oxidation of sulhydryl groups causing denaturing of a number of enzymes and from the peroxidation of membrane lipids thus increasing membrane permeability. H_2O_2 may also be as a precursor for the production of bactericidal free radicals such as superoxide (O_2^-) and hydroxyl ($OH^{\cdot}$) radicals which can damage DNA (Ammor *et al.*, 2006).

CO_2 is mainly produced by heterofermentative lactic acid bacteria. CO_2 may play a role in creating an anaerobic environment which inhibits enzymatic

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decarboxylation and the accumulation of CO_2 in the membrane lipid bilayers results in a dysfunction in permeability. CO_2 could effectively inhibit the growth of many food spoilage microorganisms, especially Gram-negative psychrotrophic bacteria. The degree of inhibition by CO_2 varies considerably between the organisms. CO_2 at 10% (v/v) could lower the total bacterial counts by 50% (v/v) and a strong antifungal activity was observed at 20–50% CO_2 (Ammor *et al.*, 2006).

Diacetyl (2, 3–butanedione, biacetyl) is produced by some species and strains of the genera *Streptococcus*, *Leuconostoc*, *Lactobacillus* and *Pediococcus* in citrate fermentation. It inhibits the growth of Gram-negative bacteria by reacting with arginine utilization (Ammor *et al.*, 2006). Diacetyl contributes to the flavor of many fermented foods. Its concentrations is reported to be 0.5–20 mg/kg in fermented dairy products, 44.0–62.5 mg/kg in bakery products and 2.9–5.2 mg/kg in some indigenous fermented foods (Lanciotti *et al.*, 2003). Sun and Oliver (1994) reported that an exposure to 50 ppm of diacetyl for 24 h was sufficient to exhibit a lethal effect on *Vibrio vulnificus*. The same concentration was able to strongly inhibit *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in the presence of a starter culture in a laboratory medium.

Among HMM compounds, bacteriocins have attracted a great interest in food industry due to their potential application in food preservation. Bacteriocins are ribosomal synthesized, extracellular released bioactive peptides or peptide complex (usually 30–60 amino acids) which has bactericidal or bacteriostatic effect on other (usually closely related) species. In all cases, the producer cell exhibits specific immunity to the action of its own bacteriocin. They generally considered to act at the cytoplasmic membrane and dissipated the proton motive force through the formation of pores in the phospholipids bilayers. Nisin is the best defined and the only purified bacteriocin produced by lactic acid bacteria that has been approved for use in food products (Cleveland *et al.*, 2001; Dunne *et al.*, 2001).

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(vi) Stimulation of immune response

Gut associated lymphoid tissue (GALT) may contact with adhesive probiotic strains and their components. Adhesion is one way of provoking immune effects. Human studies have been shown that probiotic bacteria can have positive effects on the immune system of their host (Saarela *et al.,* 2000). It has been discovered that conventional animals with a complete gut flora have increased phagocytic activity and immunoglobulin levels compared with germ-free animals. For bacteria to be effective in the process of immunostimulation, it may be necessary for them to migrate from the gut to the systemic circulation. *Lactobacilli* had been found to be capable of translocating and surviving for many days in the spleen, liver and lungs (Oyetayo and Oyetayo, 2005).

(vii) Viability and resistance during processing

Application of probiotic cultures in food products and environments represent a challenge (Kosin and Rakshit, 2006). From a processing perspective, probiotic bacteria should be technological suitable for incorporation into food systems so that they retain viability and efficacy through storage and following consumption. Candidate probiotic microorganisms must be suitable for large-scale industrial production. In this respect, the drying of live probiotic strains is a critical step in the preparation of concentrated probiotic food ingredients with most focus on freeze drying and spray drying (Corcoran *et al.,* 2006). In food processing, heating serves two purposes. High temperatures over 65 °C are highly detrimental to probiotic cultures. Lower temperatures such as a cheddarization step at 39 °C are introduced for technological purposes. Temperatures under 45 °C are generally not detrimental to probiotics. Obviously, the heating temperatures over 45 °C will destroy at least a fraction of the population depending on the temperature and the strain (Champagne *et al.,* 2005).

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1.2 Probiotic effects

Probiotics provide a number of potential health benefits mainly through maintaining good balance and composition of intestinal flora, helping to increase the body's ability to resist the invasion of pathogens and maintain the host's well being (Ranadheera *et al.*, 2010). There are several evidences supporting potential clinical applications of probiotics in the prevention and treatment of diseases. A number of health benefits in the ingestion of probiotics have been reported. These include the following:

(i) Lactose intolerance

Lactose intolerance results from insufficient activity of lactase in the human gut and causes abdominal distension, excessive flatulence and diarrhoea. Over half the world's population is unable to utilize lactose effectively (Fooks *et al.*, 1999). The lactic acid bacteria are excellent digesters of lactose due to production of β-galactosidase or phospho-β-galactosidase (O'Sullivan, 2006). It has been established that lactose administered in yoghurt can be utilized more efficiently than the same amount given in untreated milk (Fooks *et al.*, 1999; Granato *et al.*, 2010). Microbial β-galactosidase present in yogurt survives gastric passage and supports cleavage of lactose. Probiotic supplementation also modified the amount and metabolic activities of the colonic microbiota and alleviates symptoms in lactose-intolerance subjects. The changes in the colonic microbiota might be among the factors modified by the supplementation that lead to the alleviation of lactose intolerance (Baek and Lee, 2009).

(ii) Infection in the gastrointestinal tract

A number of the patients treated with antibiotics will develop antibiotic associated diarrhea (AAD) because their intestinal flora responsible for the natural colonization resistance is disturbed or reduced (Mercenier *et al.*, 2003). The bacterial agent most commonly detected in AAD is *C. difficile, Klebsiella oxytoca,*

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multidrug resistant *Salmonella*, *C. albicans* and *C. tropicalis*. Mercenier *et al.* (2003) revealed that *Bifidobacteria* exhibited positive evidences against AAD. The protection mechanism by this probiotic has not been fully established. However, one or more of the following are possible: competition of nutrients, secretion of antimicrobial substances, reduction of gut pH through SCFA formation, blocking of adhesion sites, attenuation of virulence, blocking of toxin receptor sites, immune stimulation and suppression of toxin (Fooks *et al.*, 1999). The efficacy and safety of a fermented milk combining *L. acidophilus* and *L. casei* is widely available in the preventing AAD. It could be indicated that the daily administration of *Lactobacilli*-fermented milk was safe and effective in the prevention of AAD in hospitalized patients (Hickson *et al.*, 2007). Consistent with Agarwal and Bhasin (2002), receiving fermented milk with 10^8 CFU/g of *L. casei* DN 114001, *L. bulgaricus* and *S. thermophilus* shortened the duration of diarrhoea significantly ($P < 0.05$) (Agarwal and Bhasin, 2002). Furthermore, the reduction of acute diarrhoea in children from enterotoxigenic *E. coli* (ETEC) by *L. acidophilus* LB was investigated. *L. acidophilus* LB added to the oral rehydration therapy during acute diarrhoea reduced the duration of diarrhoea in treated children compared to the placebo group (Simakachorn *et al.*, 2000).

(iii) Suppression of cancer

Colon cancer is a multi-factorial and complex neoplasm involving both genetics and environmental factors. There seems to be a strong relationship between colon cancer, diet and intestinal microflora. The rupture of the intestinal microflora equilibrium due to a bad diet seems to be related to an increase in the risk of developing colon cancer (Iannitti and Palmieri, 2010). Certain bacterial species in the colon produce harmful substances that seem to be correlated to cancer (Liong, 2008). Bacterial enzymes converting precarcinogens to active carcinogens are produced in the gut but their involvement in the pathogenesis of cancer is unclear (Saarela *et al.*, 2000). It has been thought that probiotics may modulate several major intestinal functions potentially associated with the development of colon cancer preventing the growth of deleterious organisms, producing anticarcinogenic substances and moving the balance of gut bacteria in favour of the ones beneficial for

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the organism. In the past few years, Liong (2008) underlined the different properties attributed to probiotics and prebiotics such as anti-carcinogenic, antimutagenic properties, ability of modifying differentiation processes in tumor cells, production of SCFAs and alteration of tumor gene expressions. According to Takeda and Okumura (2007), daily intake of *L. casei* strain Shirota provided a positive effect on the activity of NK cells which seem to exert a key role in protecting the human organism against cancer. Lee and Salminen (2009) suggested that *L. acidophilus* fed to healthy volunteers displayed a significantly decrease in β-glucoronidase, nitroreductase and azoreductase activities. Moreover, animal models have shown that dietary intake of lyophilized cultures of *B. longum* significantly suppressed the development of azoxy methane-induced aberrant cryptic foci (ACF) formation in the colon. This was confirmed by another group which used the animal models to determine that a combination of *B. longum* and the prebiotic inulin was effective in generating beneficial changes related to tumour risk.

(iv) Coronary heart disease

Hypercholesterolemia (elevated blood cholesterol levels) is considered a major risk factor for the development of coronary heart disease and although pharmacologic agents (such as statins or bile acid sequestrants) are available to treat this condition. However, these agents are often suboptimal and expensive and can have unwanted side effects. The cholesterol-lowering effects could be partially ascribed to bile salt hydrolase (BSH) activity. Deconjugated bile salts are less efficiently reabsorbed than their conjugated counterparts, which results in the excretion of larger amounts of free bile acids in feces. Also, free bile salts are less efficient in the solubilization and absorption of lipids in the gut. Therefore, deconjugation of bile salts could lead to a reduction in serum cholesterol either by increasing the demand for cholesterol for de novo synthesis of bile acids to replace those lost in feces or by reducing cholesterol solubility an thereby absorption of cholesterol through the intestinal lumen (Begley *et al.*, 2006). Several studies have reported on the ability of probiotic strains to lower serum cholesterol, directly assimilate cholesterol or produce metabolite affecting the systemic levels of blood

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lipids. Adlay-soymilk fermented with *L. plantarum* or *L. paracasei* significant decreased ($P < 0.05$) serum cholesterol level and ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol in hamsters (Wang *et al.*, 2010). Consistent with Sirilun *et al*. (2010), *L. plantarum* TGCM 15 and TGCM 33 exhibited the significantly ($P < 0.05$) highest cholesterol-lowering activity with the percentage more than 50%. Moreover, BSH-active *Lactobacilli* were investigated for cholesterol reduction in pigs. Probiotic containing *L. johnsonii* and *L. reuteri* showed a lowering effect on serum cholesterol from 3.25 to 2.74 mmol/L after 3 weeks probiotic feeding (du Toit *et al.*, 1998).

(v) Immune stimulation

The effects of probiotics on the immune system such as induction of pro- and/or anti-inflammatory cytokines, elevation of serum level of IL-10, induction of maturation of dendritic cells (DC), enhancement of serum antibody response to orally and systemically administered antigens, enhancement of the immunoreactivity of spleen cells and phagocytes, activation of the gene for human beta defensin 2 in intestinal mucosa and induction of oral tolerance to β-lactoglobulin (Ljungh and Wadstrom, 2006).

Intestinal epithelial cell are indirect contact with microbiota in the gut lumen and also interface and segregate the immune system. Intestinal bacteria can bind to recognition receptors expressed on the surface of epithelial cells and thus trigger a cascade of immunological defense mechanisms such as the production of pro and anti-inflammatory cytokines. The innate immune system recognizes a large group of conserved molecular structures in bacteria including lipopolysaccharides (LPS), lipoteichoic acid and unmethylated CpG motifs of DNA and is able to distinguish between own and foreign structures. Probiotic bacteria have also been found to activate the key elements responsible for the formation of pro-inflammatory cytokines and chemokines. Probiotic bacteria can enhance the adaptive immune response and antibody formation (Saxelin *et al.*, 2005). In addition, there is sufficient evidence suggesting that probiotics specially (e.g. antibody production, cytokinase production,

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lymphocyte proliferation, delayed-type hypersensitivity) and nonspecifically (e.g. phagocyte function, NK cell activity) modulate the host's immune system. Interestingly, probiotics given to pregnant women reduced the incidence of atopic disease in the breast-fed infants (Rautava *et al.*, 2002).

1.3 Application of probiotics in non-dairy products

Probiotics have been successfully incorporated in a number of foods including the traditional vehicles of fermented milks, yoghurt, fruit, cereals and vegetables products (Yoon *et al.*, 2004; Nicolescu and Buruleanu, 2010; Ranadheera *et al.*, 2010; Rivera-Espinoza and Gallardo-Navarro, 2010). Probiotic products are usually marketed in the form of fermented milks and yoghurts. However, the allergy to dairy products affects negatively some persons. Lactose intolerance and the cholesterol content are two major drawbacks related to the fermented dairy products. Traditions and economic reasons that limit the use of dairy fermented products in some developing countries promote the idea of reduction of milk components as vehicles for the probiotic agents (Prado *et al.*, 2008). For these reasons, the growing demand for new probiotic foods has stimulated the development of non-dairy products (Yoon *et al.*, 2006; do Espírito Santo *et al.*, 2011).

Currently, technological advances have made possible to alter some structural characteristics of fruits and vegetables matrices by modifying food components in a controlled way. This could make them ideal substrates nutrients such as mineral, vitamins, dietary fibers and antioxidants, while lacking the dairy allergens that might prevent consumption by certain segments of the population (Yoon *et al.*, 2005). Although lactic acid bacteria have been considered a difficult microorganism that demands various essential amino acids and vitamins for growing, it has been established that some of lactic acid bacteria were able to grow in fruits and vegetables matrices. The pomegranate juice was proved to be a suitable media for production of a fermented probiotic drink. Normally, Pomegranate (*Punica granatum*, Punicaceae) is known to have considerable health-promoting properties with antimicrobial, antiviral, anticancer, antioxidant and antimutagenic effects. Mousavi *et al.* (2011)

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revealed that *L. plantarum* DSMZ 20174, *L. delbrueckii* DSMZ 20006, *L. paracasei* DSMZ 15996 and *L. acidophilus* DSMZ 20079 had a better growth rate in the pomegranate juice with the viable cell counts of > 8 log CFU/mL. During 2 weeks storage, *L. plantarum* DSMZ 20174 and *L. delbrueckii* DSMZ 20006 were capable to survive well at an acceptable level (> 6 log CFU/mL), whereas *L. paracasei* DSMZ 15996 and *L. acidophilus* DSMZ 20079 lost their viability during cold storage (4 °C).

Furthermore, orange and pineapple juices could be used as substrates for the growth of *L. casei*, *L. rhamnosus* and *L. paracasei*. All strains exhibited a great survival at levels of > 7 log CFU/mL in orange juice and > 6 log CFU/mL in pineapple juice for at least 12 weeks (Sheehan *et al.*, 2007). The feasibility of noni (*Morinda citrifolia*) as a raw substrate for the production of probiotic noni juice by lactic acid bacteria (*L. casei* BCRC 17002 and *L. plantarum* BCRC 10069) and *Bifidobacteria* (*B. longum* BCRC 14602) was reported. All strains grew well on noni juice and reached nearly 9 log CFU/mL after 48 h of fermentation at 30 °C. After 4 weeks of cold storage at 4 °C, *L. plantarum* BCRC 10069 and *B. longum* BCRC 14602 remained the viability at ~5 log CFU/mL. In contrast, *L. casei* BCRC 17002 was unable to survive at low pH or high acid conditions in fermented noni juice and the completely loss of viability was observed after 3 weeks. Moreover, noni juice fermented by *B. longum* BCRC 14602 had a high antioxidant capacity that did not differ significantly ($P < 0.05$) from that of lactic acid bacteria (Wang *et al.*, 2009).

Interestingly, fruits also have been proposed as immobilization supporters for probiotics. The applicability of apple as an ingredient to improve probiotic viability in lactic acid production process was investigated. Pieces of apple were shown to offer a proper material for *L. casei* immobilization instead of inorganic supports such as alginates, ceramic beads or porous glass. *L. casei* cell immobilized on apple pieces were used for 15 repeated batch fermentations of whey resulted in higher values of lactic acid production yield of 34.1, 49.0 and 62.7 g/100 g at 30, 37 and 45 °C, respectively (Kourkoutas *et al.*, 2005). Moreover, the direct fermentation of banana puree as the medium by immobilized *L. acidophilus* BCRC 10695 was

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determined to develop a novel product with symbiotic effects. The cell concentration of *L. acidophilus* BCRC 10695 immobilized in gel beads was found to be 10^8 CFU/(mL gel) higher than and that of the free cell fermentation (10^6 CFU/mL) (Tsen *et al.*, 2003).

Vegetables are strongly recommended in the human diet since they are rich in antioxidant, vitamins, dietary fibers and minerals. The major part of the vegetables consumed in the human diet are fresh, minimally processed, pasteurized or cooked by boiling in water or microwaving would bring about a number physical characteristics and chemical composition changes of vegetables. Fermentation by lactic acid bacteria may be considered as a simple and valuable biotechnology for maintaining or improving the safety, nutritional, sensory and shelf-life properties of vegetables (Rodríguez *et al.*, 2009). Strains of species belonging to *L. plantarum* and *Leuconostoc mesenteroides* are the most common bacteria in natural vegetable lactic acid fermentation. Furthermore, *L. paracasei*, *L. casei*, *L. delbrueckii* and *L. brevis* were also found in vegetables. According to Yoon *et al*. (2004), *L. plantarum* C3, *L. casei* A4, *L. acidophilus* LA39 *and L. delbrueckii* D7 were capable of rapidly utilizing tomato juice for cell synthesis and lactic acid production without nutrient supplement and pH adjustment. The viable cell counts of all strains were greater than 9 log CFU/mL after 72 h fermentation. The pH of the culture reduced to 4.1 or below and the acidity increased to more than 0.65%. The viable counts of 4 strains of lactic acid bacteria in fermented tomato juice ranged from 6 to 8 log CFU/mL after 4 weeks of cold storage at 4 °C. It could be indicated that probiotic tomato juice could serve as a health beverage for vegetarians or consumers who are allergic to dairy products.

In addition, cabbage, a cruciferous vegetable, is rich in mineral, vitamin C and dietary fibers. Yoon *et al*. (2006) used the same lactic acid bacteria (*L. plantarum, L. casei* and *L. delbrueckii*) to evaluate the suitability of cabbage as raw material for the production of probiotic cabbage juice. All strains were able to grow rapidly on sterilized cabbage juice without nutrient supplementation and reached nearly 8 log CFU/mL after 48 h of fermentation at 30 °C. *L. plantarum* and *L. delbrueckii* produced significantly more titratable acidity (1%) expressed as lactic acid than *L.*

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casei (0.74%). Also, beetroot were evaluated as a potential substrate for the production of probiotic beet juice by lactic acid bacteria (Rakin *et al.*, 2007;Yoon *et al.*, 2005). *L. plantarum* C3, *L. acidophilus* LA39, *L. casei* A4 and *L. delbrueckii* D7 were found capable of rapidly utilizing beet juice for cell synthesis and lactic acid production. Especially, *L. plantarum* C3 and *L. acidophilus* LA39 produced a greater amount of lactic acid than other cultures and reduced the pH of fermentation beet juice from an initial value of 6.3 to below 4.5 after 48 h of fermentation at 30 °C (Yoon *et al.*, 2005).

Additionally, the enrichment of the fruit juice-based medium with nutritive substances has also been studied. The beetroot and carrot juices enriched with brewer's yeast autolysate were subjected to lactic acid fermentation with *L. acidophilus* NCDO1748 in order to improve their nutritive values. Brewer's yeast autolysate contributed to the increase in the number of viable cells of *L. acidophilus* NCDO1748 (8–9 log CFU/mL) and production of lactic acid (3.42 g/L) in comparison with fermentation of the beetroot and carrot juices without brewer's yeast autolysate. It could be indicated that fermentation of vegetable juices enriched with yeast autolysate could increase the nutrition including the amino acid, mineral content, vitamins and antioxidant activity (Rakin *et al.*, 2007).

Interestingly, lactic acid fermentation could increase folate concentrations in vegetables making foods. Commercial starter cultures aimed for manufacture of fermented dairy products were subjected to mixtures of granted and blanched root vegetables mainly beetroots and turnips. *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Bifidobacterium and Propionibacterium* grew well in the different vegetable mixtures. The final pH varied between 3.3 and 3.5 and bacterial number increased to 8×10^8–2×10^9 CFU/mL. Mix starter cultures no. 1 (*L. plantarum* + *Lc. cremoris* + *Lc. lactis* + *Leuconostoc* spp.) was found to produce the highest amounts of 5-methyl-tetrahydrofolic acid (5-CH_3-H_4 folate) resulting in almost a doubling of bioavailable folate form mainly as 5-CH_3-H_4 folate (247 µg/kg wet weight) (Jägerstad *et al.*, 2004). In carrot juice, lactic acid fermentation was also shown to improve

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mineral solubility to different extents at simulated physiological conditions: Mn (2-fold); Fe (1.5–1.7-fold); Zn (1.2-fold); Cu (1-fold) (Bergqvist *et al.*, 2005).

Cereal grains are considered to be one of the most important sources of protein, carbohydrates, vitamins, minerals and fiber for people all over the world (Blandino *et al.*, 2003). Cereals can be used as sources of non-digestible carbohydrates in order to promote several beneficial physiological effects and stimulate the growth of *Lactobacilli* and *Bifidobacteria* present in the colon acting as prebiotic. Furthermore, cereals can be used as encapsulated material for probiotic in order to enhance their stability (Gobbetti *et al.*, 2010). Therefore, cereals may represent a cheap way to obtain a rich substrate that sustains the growth of beneficial probiotic (Cheng *et al.*, 2005; Rivera-Espinoza and Gallardo-Navarro, 2010).

More studies are being done to demonstrate that cereals are suitable substrates for the growth of some probiotic bacteria. In the last few decades, soybean has received attention from the researchers because of its protein quality and their functional properties which has a great potential application in the food industry. For example, soymilk is suitable for the growth of the lactic acid bacteria (Pyo *et al.*, 2005; Tang *et al.*, 2007; Bamrungna, 2009; Yeo and Liong, 2010), especially *Bifidobacteria* (Chou and Hou, 2000; Hou *et al.*, 2000). Consumption of fermented soymilk is beneficial to the ecosystem of the intestinal tract by increasing the population of probiotic (*Bifidobacterium* spp. and *Lactobacillus* spp.) and reducing the population of *Clostridium perfringens* and coliform in fecal samples. According to Cheng *et al.* (2005), the ratios of *Bifidobacterium* spp. and *Lactobacillus* spp. to *C. perfringens* increased and the population of coliform organisms decreased significantly ($P < 0.05$) during the period of fermented soymilk consumption. This may be because soybeans contain certain types of oligosaccharides such as raffinose and stachyose that can be utilized by *Lactobacillus* spp. as energy sources. Furthermore, soymilk fermentation could reduce the beany odor and gas production in the intestine. A higher percentage of survival was also mentioned when the dried fermented soymilk was stored at 4 °C than 25 °C. Chou *et al*. (2000) reported that soymilk could support the growth of *B. infantis* CCRC 14633 and *B. longum* B6 with

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the viability of 8.5 and 7.1 log CFU/mL, respectively, after fermentation for 48 h. Moreover, the addition of yeast extract, peptone, tryptone, casitone or N-Z-Case plus to soymilk enabled *B. infantis* CRC 14633 to reach its maximum population (~8.7 log CFU/mL) in a shorter cultivation time of 24 h. The population of *B. infantis* CCRC 14633 and pH reduced more in fermented soymilk drink when held at 25 °C than 5 °C. After 10 days of storage, viable cell count and pH decreased 2.0 log CFU/mL and pH 0.4, respectively. Yeo and Liong (2010) revealed the production and use of the fermented soymilk drinks as probiotic, mainly soybean yoghurt, can be supplemented with fructooligosaccharides (FOS) and inulin. *Lactobacillus* sp. FTDC 2113, *L. acidophilus* FTCD 8033, ATCC 4356, *L. casei* ATCC 393 and *B. longum* FTDC 8643 showed the viability exceeding 7 log CFU/mL in prebiotic-supplemented soymilk after 24 h. Furthermore, supplementation with FOS also increased the α-galactosidase activity of probiotics leading to enhanced hydrolysis and utilization of soy oligosaccharides.

Probiotic strains could metabolize deconjugation of isoflavones in soybean (King *et al.*, 1996). Normally, isoflavones occur in the form of aglycones and their corresponding glucosidic conjugates. Isoflavone aglycones have associated with the prevention and potential treatment of hormone-dependent disorders based on epidemiological. The anti-cancer function of soybean isoflavones was shown to be associated with genistein which inhibits protein tyrosine kinases, DNA topoisomerase and binds weakly to estrogen receptors. On the other hand, the glucoside isoflavones are very poorly absorbed in the small intestine as compared with their aglycones because of their greater molecular weight and higher hydrophilicity of the glucoside (Chang and Nair, 1995). The glucoside conjugates of isoflavones were converted to isoflavones aglycones during soybean processing (Toda *et al.*, 2001). *Lactobacilli* and *Bifidobacteria* play major roles in the intestinal hydrolysis of numerous plants β-glucosides (Otieno *et al.*, 2007a; Otieno *et al.*, 2007b; Wei *et al.*, 2007). According to Pyo *et al*. (2005), *L. plantarum* KFRI 00144, *L. delbrueckii* subsp. *lactis* KFRI 01181, *B. breve* K-101 and *B. thermophilum* KFRI 00748 have potential as a functional starter culture for developing fermented soymilk with higher estrogenicity and better

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absorption facilitating the bioavailability of isoflavones. This result collaborates to those of Wei *et al*. (2007) that the isoflavone aglycone had a significant increase of 62–96% in fermented soymilk compared to 17% in non-fermented soymilk ($P < 0.05$).

Oat is one of the major sources of β-glucan which is recognized as the main functional component of cereal fiber. It has been reported that *L. reuteri*, *L. acidophilus* and *B. bifidum* grew well in oat-based substrate (Mårtenson *et al.*, 2002). Furthermore, fermented whole-grain oat with *L. plantarum* B28 was studied in order to produce fermented drink combined the health benefits of a probiotic culture with the oat prebiotic β-glucan. The results revealed that the viability of *L. plantarum* was 10 log CFU/mL after refrigerated storage (4–6 °C) for 24 days. The addition of sweeteners aspartame, sodium cyclamate saccharine and Huxol (12% cyclamate and 1.2% saccharine) had no effect on the dynamics of the fermentation process and on the viability of the starter culture during product storage. β-glucan content in the drink (0.31–0.36%) remained unchanged both throughout fermentation and storage of the drink. The shelf life of the symbiotic oat drink under refrigerated temperature was estimated to be 21 days (Angelov *et al.*, 2006). Additionally, Yosa, a new oat-based fermented food containing *Lactobacilli* and *Bifidobacteria*, was developed due to its functionality such as oat fiber, β-glucan, lactose-free, low in fat and probiotics suitable for vegetarian (Blandino *et al.*, 2003). Moreover, the feasibility of culturing probiotic in solid-state fermentation using oat bran as nutrient sources was studied. It was found that *L. plantarum* NCIMB 8826 cell population increased to an average of 2.6×10^9 CFU/g dry solids in the fermentation undertaken on oat bran hydrolyzed using enzymes from *Aspergillus oryzae* (Patel *et al*., 2004b).

Maize is the most important sources of food for millions of people, particularly in Latin America and Africa. Maize is promising substrate lacking of gluten protein, a major source of concern in baked goods from wheat and other cereals, in order to avoid celiac disease. Fairly successful attempts have been made to develop sour maize bread using the sourdough technique. Edema and Sanni (2008)

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identified microorganisms from fermented maize meal. They reported that *L. plantarum, L. brevis, L. fermentum, L. acidophilus, P. acidilactici, L. mesenteroides, L. dextranicum* and *S. cerevisiae* are the dominant starter cultures presence until the end of fermentation. The viable cell counts of lactic acid bacteria and yeast were found to increase steadily from approximately 4.2 to 6.6 log CFU/mL.

The growing demand for new non-dairy probiotic food products worldwide has stimulated (do Espírito Santo *et al.*, 2011). Proviva®, the first probiotic food that does not contain milk, comprises *L. plantarum* 299v fermented oatmeal gruel was developed. This product contains 5% of the oat meal gruel mixed with a fruit drink. Malted barley is added to increase the liquefaction of product and *L. plantarum* 299v carries out the fermentation. The final viable cell count of *L. plantarum* 299v is 1×10^{10} CFU/mL (Molin, 2001). Additionally, *Attune Foods*™, probiotic chocolate bars, contains *B. lactis, L. acidophillus* and *L. casei* (6×10^{9} CFU/g) and Kashi® Vive™, new probiotic cereal product, has gained more interest for many health conscious consumers. Therefore, it could be indicated that cereals could influence growth, viability and survival, acid and bile tolerance and different functionality of probiotics that determine their efficacy in gastrointestinal tract. Thus, careful investigation of the interaction of different probiotics and food components should be considered in developing functional probiotic foods.

2. Spray drying of lactic acid bacteria

Spray drying is a common unit operation to convert liquid materials into powders for preservation, ease of storage, transport and handling and other economic considerations. The main advantages of spray drying processes over other drying processes are listed in Table 3. The preservation of lactic acid bacteria by spray drying has been widely studied as an alternate industrial process for the preservation of lactic acid starter cultures because of the high costs and energy consumption of freezing and freeze drying process (Riveros *et al.*, 2009). The successful spray drying of probiotic has been reported for a number of different strains including *L. plantarum, L. rhamnosus* GG, *L. acidophilus, S. thermophilus, L. paracasei, L.*

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salivarius, B. longum and *B. infantis* (Teixeira *et al.*, 1996; Wang *et al.*, 2004; Bucio *et al.*, 2005; Kearney *et al.*, 2009; Golowczyc *et al.*, 2011a; Sunny-Roberts and Knorr, 2011). Some studies on spray drying of lactic acid bacteria are summarized in Table 4.

Table 3 Key advantages of spray drying.

No.	Key advantages of spray drying
1.	Dried products of predetermined characteristics (size, density, moisture content and nutrients content) and types (fine powders, granules and agglomerates) can be produced.
2.	Powder quality remains constant throughout the entire production run when drying conditions are held constant.
3.	Powders with a narrow size distribution can be achieved.
4.	Dried products are ready for packaging - no additional grinding is required
5.	Both heat-sensitive and heat-resistive liquid materials can be processed without a significant damage to the product.
6.	Heat-spoilage to the product is relatively small due to short exposure times in a hot environment, cooling effects in a critical drying period and also due to the solvent removal at temperatures lower than the normal boiling point.
7.	Versatile process - same equipment can be used to dry a wide range of liquid materials.
8.	Continuous operation - high production rate (over 25 tons powder/h/drier) - economical process.
9.	Operation and maintenance of the plant can be fully automated.
10.	Vast knowledge has been established to characterize different phenomena of spray drying using various mathematical models and computational tools.

Source: Bhandari *et al*. (2008)

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Table 4 Summary of some studies on the spray drying of lactic acid bacteria.

Microorganisms	Survival (%)	Temperature (°C)		Carriers	References
		Inlet	Outlet		
L. kefir CIDCA 8348	98%[a]	160	70	11% skim milk	Golowczyc *et al.* (2011)
L. kefir CIDCA 8321	96%[a]				
L. plantarum CIDCA 83114	96%[a]				
L. rhamnosus GG	98%[a]	–[b]	65–70	20% trehalose	Sunny-Robert and Knorr (2011)
L. paracasei NFBC 338	89%[a]	170	80–85	Fermented yoghurt	Kearney *et al*. (2009)
L. delbrueckii subsp. *bulgaricus*	68%[a]				
S. thermophilus	85%[a]				
L. plantarum CFR 2191	100%	140	40	10% skim milk	Reddy *et al.* (2009)
L. salivarius CFR 2158	100%				
P. acidilactici CFR 2193	97%				

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Table 4 (Continued)

Microorganisms	Survival (%)	Temperature (°C)		Carriers	References
		Inlet	Outlet		
L. acidophilus	91%[a]	100	58	skim milk	Riveros *et al.* (2009)
L. rhamnosus GG	70%[a]	–[b]	70	20% skim milk	Anata *et al.* (2005)
L. reuteri KUB-AC5	88%[a]	170	85	20% skim milk	Hamsupo *et al*. (2005)
B. longum B6	89% [a]	100	50–60	10% gum arabic	Lian *et al*. (2002)
	98% [a]			10% gelatin	
	95% [a]			10% soluble starch	
	99% [a]			10% skim milk	

[a] calculated from report value

[b] the results was not reported

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Spray drying involves formation of droplets from the bulk liquid followed by the removal of moisture from the liquid droplets. The material in the liquid state is sprayed in the drying chamber where the low-humidity hot gas (drying gas/medium) is mixed with the dispersed droplets. The spray of individual droplets is produced by the rotary wheel/disc atomizers, pressure nozzle or pneumatic-type atomizers. Spraying or atomization produces 10 to 500 µm droplets. The moisture in the form of vapor quickly evaporates from the suspended droplets due to simultaneous and fast heat and mass transfer processes. Drying of the droplets continues inside the drying chamber until the desired particle characteristics are achieved (Bhandari *et al.*, 2008). A typical spray drier should consist of at least four main components including heating system, atomization system, drying chamber and powder seperators (Figure 2).

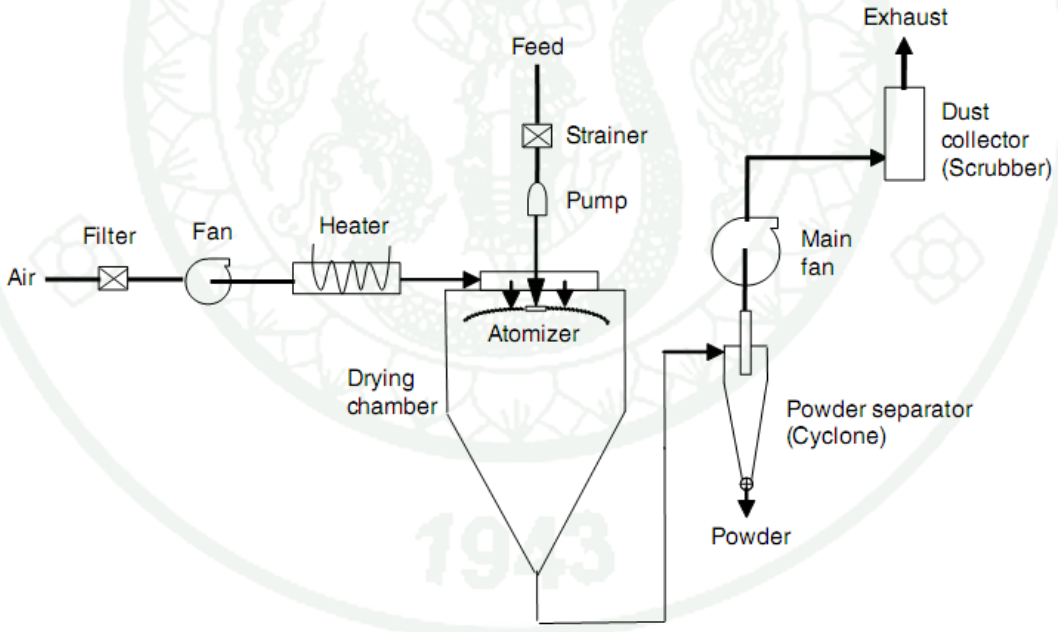

Figure 2 A single-stage spray drying system.

Source: Bhandari *et al*. (2008)

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2.1 Cellular inactivation during spray drying

During spray drying process, the drying material generally undergoes a decrease in water activity and an increase in temperature. Cellular injury of microorganisms resulted in a loss of viability (Ananta, 2005; Ananta *et al.*, 2005). The removal of free water seems to have less detrimental effect on the viability of cells (Santivarangkna *et al.*, 2007). Desiccation imposes severe stress on microorganisms as the removal of water induces conformational changes in protein and cell membrane (Crowe *et al.*, 1992). The cell membrane is among the most susceptible sites in bacterial cells to the stresses associated with spray-drying, while the cell wall, DNA and RNA are also known to be affected leading to loss of metabolic activity (Teixeira *et al.*, 1995b). Removal of hydrogen-bonded water from the headgroup region of phospholipid bilayers increases the headgroup packing and forces the alkyl chains together. As a result, the lipid component may undergo a transition from lamellar to gel phase which can be seen as a dehydrated lamella phase in which the chains are stiff and fully extended. Additionally, certain phospholipids undergo a transition from lamellar to hexagonal phase as water is removed (Leslie *et al.*, 1995).

To understand the cause of damage to membranes from drying, it is necessary to understand the organization of membrane phospholipids, especially, the lamellar fluid crystalline bilayers which form the framework and is the biologically active state of the bacterial cytoplasmic membrane. It is crucial for the cell to maintain its fluidity and structure of the membrane because changes in membrane not only affect membrane function but also the function of embedded membrane proteins (Santivarangkna *et al.*, 2008a). There is evidenced that a number of cell components such as DNA, RNA and protein would be inflicted when cells are dried to low-water content. Leakage of cell membrane was indirectly testimony by the increase in the sensitivity of cells to NaCl or by increase in intracellular enzyme lactate dehydrogenase in the surrounding medium after rehydration (Selmer-Olsen *et al.*, 1999).

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A well-known theory for heat inactivation was proposed by Gould (1989). Heat is assumed to inactivate a critical component. Loss of the less critical components does not cause death until their numbers are reduced to very low numbers or the cell is subjected to additional stress (Figure 3a-d). Schematic drawing shows the lethal and sublethal injuries of cells based on the critical target theory (Gould, 1989). From Figure 3a, the critical component (large filled circle) exists in only one or relatively few copies per cell. The less critical components (small open circles) exist in high copy numbers. Sublethal injured cells survived heating are able to grow in media when the critical component remains intact. Figure 3b reveals that dead or lethally injured cells become irreversibly killed when the critical component is inactivated by heat beyond repair or the less critical components are reduced to a very low number (Figure 3c). Sublethal injured cells may fail to resume growth when subjected to secondary stress such as osmotic stress, low pH, etc (Figure 3d).

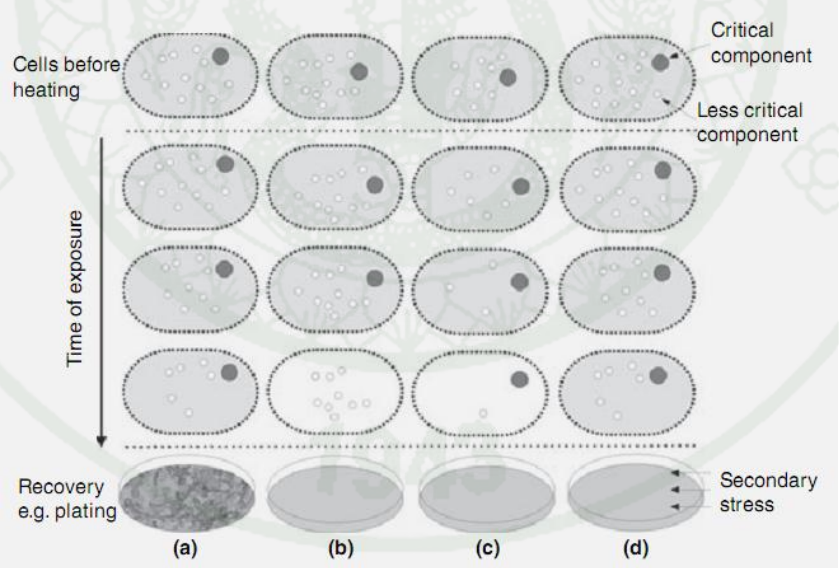

Figure 3 Schematic drawing of the lethal and sublethal injuries of cells.

Source: Santivarangkna *et al*. (2008b)

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Among those susceptible cell components, ribosomes have been speculated to be the critical component for heat inactivation. Santivarangkna *et al*. (2008b) reviewed that the temperature at which the death rate determined by viable cell count is maximal, coincides with the thermogram peak resulted from denaturation of the 30S ribosomal subunit. While, the denaturation of DNA requires a temperature higher than that for killing cells and does not correlate with the thermal stability of bacteria. However, it should be remarked that ribosomes account for a large proportion of the cell's dry matter and their denaturation is highly enthalpic. The largest endothermic peak caused by ribosomal denaturation may conceal other less energetic calorimetric events such as the unfolding of essential proteins, e.g. alpha and beta subunits of RNA polymerase. The critical components should have essential functions which are provision of the genetic codes required to make the proteins or nucleic acids that are essential for a viable cell. A critical component was redefined as a component whose number of functioning copies within the cells must remain above a critical level for the cell to survive or reproduce. For example, ribosomes will be a critical component if heat damages a number of ribosomes beyond a critical level that the remaining functioning copies of ribosomes cannot cope with the required essential function, i.e. protein synthesis. Besides ribosomes, critical components can also be cell envelopes, DNA and RNA polymerase. Furthermore, it is not necessary that the same critical component is lost in all cells and death may be due to the destruction of more than one critical component.

2.2 Factors affecting viability of lactic acid bacteria during drying

The industrial exploitation of lactic acid bacteria as starter or probiotic cultures depends strongly on the preservation technologies employed which is required to guarantee long-term delivery of stable cultures in terms of viability and activity. Maximization of survival of lactic acid bacteria cultures during drying and subsequent storage for long periods is of vital importance (Carvalho *et al.*, 2004). Several factors affect the survival of bacteria during spray drying such as growth phase and growth media, inlet and outlet temperatures, protective agents and choice of strains.

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2.2.1 Type of strain

Distinct strains of the same species could differ in their behaviors during drying. In general, *Bifidobacteria* are more labile to spray drying than *Lactobacilli*. Lian *et al*. (2002) reported that *B. longum* survived better than *B. infantis* after spray drying. According to Picot and Lacroix (2004), *B. breve* R070 was more labile than *B. longum* R023. *B. longum* R023 exhibited 25.7% survival (1.7×10^9 CFU/g) compared to those of *B. breve* R070 which displayed the survival rate only 1.4% (8.6×10^7 CFU/g) after during spray drying at outlet temperature of 80 °C (Picot and Lacroix, 2004). Also, Sunny-Robert and Knorr (2009) reported that the survival rate of *L. rhamnosus* LGG was 69% higher than that of *L. rhamnosus* E800 (23% survival) after spray-drying.

Additionally, viability is correlated to cell morphology such as cell size and shape (Santivarangkna *et al.*, 2007). Bozoğlu *et al.* (1987) reported that small spherical *Streptococci* cells are more resistant to freeze drying than long rod *Lactobacilli* cells. Consistent with Strasser *et al.* (2009), *Enterococci* are apparently more resistant to freeze drying than rod-shaped *Lactobacilli* which are more susceptible to membrane damage due to their higher surface area (Fonseca *et al.*, 2000; Fonseca *et al.*, 2004). This was also supported by Bielecka and Majkowska (2000) that *S. thermophilus* MK-10 exhibited survival of 54.7% (5.2×10^8 CFU/mL) higher than *L. delbrueckii* subsp. *bulgaricus* 151 (15.8% survival, 1.5×10^8 CFU/mL) during spray drying yoghurt (outlet temperature of 70 °C). In contrast, *S. lipolytica* CIDCA 812 showed the lowest survival rate of approximately 75.4% (6 log CFU/g) lower than those of *L. plantarum* CIDCA 83114 (90.9% survival) and *L. kefir* CIDCA 8348 (84.1% survival). Furthermore, the tolerance to thermal and mechanical stresses is the greatest in Gram-positive bacteria followed by yeast and the lowest in Gram-negative bacteria possibly due to their thin cell was structure (Donsi *et al.*, 2009).

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2.2.2 Growth phase and media

Growth phase has been demonstrated to be an important factor affecting the survival of lactic acid bacteria. The optimal harvested time of cells for starter culture production depends on the specific organism. It has been generally accepted that cells harvested at the stationary phase during a batch culture showed the highest stress tolerance compared to those harvested at the lag and exponential phase (Meng *et al.*, 2008; Fu and Chen, 2011; Peighambardoust *et al.*, 2011). This was supported by Teixeira *et al*. (1995b) that cells at stationary phase are significantly ($P < 0.05$) more resistant than cells at exponential phase. Furthermore, over 50% survival (2.9×10^9 CFU/g) was obtained when *L. rhamnosus* GG was spray-dried at stationary phase. While, early log phase and lag phase exhibited 14 and 2% survival, respectively (Corcoran *et al.*, 2004). It could be indicated that bacteria that enter into stationary phase develop a general stress resistance and thus more resistant to various types of stresses (including subsequent down-stream processing and storage) than bacteria in the log phase due to carbon starvation and exhaustion of available food sources that trigger stress responses to allow survival of the cell population (Morgan *et al.*, 2006; Toledo *et al.*, 2010).

Moreover, culture medium compositions were found to affect on the cell survival during the subsequent drying process (Fu and Chen, 2011). During drying process, cells are subjected to conditions of low water activity. The accumulation of compatible solutes would be expected to enhance survival during the process (van de Guchte *et al.*, 2002). Compatible solutes are small organic molecules sharing a number of properties: (i) they are very soluble and can be accumulated to high levels in the cytoplasm of osmotically stressed cells, (ii) they are either neutral or zwitterionic molecules and specific transport system available in the cytoplasmic membrane allow the controlled accumulation of these compounds and (iii) they do not alter enzyme activity and may even protect enzymes from denaturation (Carvalho *et al.*, 2004). There are many kinds of compatible solutes such as amino acids, betaine, glucosyl glycerol, polyols, carnitine, mannosylglycerate and non-reducing sugar disaccharide which can be accumulated as end products of metabolism (Prasad *et al.*,

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2003; Chapa, 2006; Morgan *et al.*, 2006). Their accumulations within a cell protects the cell from the osmotic stress during dehydration which is achieved by balancing the osmotic different between the interior and exterior of the cell membrane. These can also help to stabilize proteins and the cell membrane during osmotic stress conditions (Morgan *et al.*, 2006).

Moreover, the accumulation of these compatible solutes also displayed cross-protection effects towards other stresses such as heat stress (Silva *et al.*, 2011). Accumulation of compatible solutes has been shown to be associated with increased thermotolerance of various microorganisms. According to Tymczyszyn *et al*. (2007), MRS supplemented with sucrose (MRS-sucrose), or polyethylene glycol (MRS-PEG) could enhance the viability of *L. delbrueckii* ssp. *bulgaricus* CIDCA 333 after drying of approximately 1 log MPN/mL compared to no supplemented MRS. Pre-stressed of *L. rhamnosus* HN001 (DR20) with salt (0.6 M NaCl) improved the viability (2 log reduction) compared with the non-stressed control (7.3 log reduction) after storage at 30 °C in the dried form (Prasad *et al.*, 2003). Furthermore, the growth of *Lactococcus lactis* subsp. *lactis* C10 at low water activity (2% w/v NaCl) correlated with the ability to accumulate glycine betaine (O'Callaghan and Condon, 2000). Similarly, the strains expressing *bet*L gene exhibited a significant increase in resistance to several stresses. The survival rate after spray drying of *L. salivarius* UCC118 (*bet*L^+) grown under low water activity solute was 1.4% higher than that of *L. salivarius* UCC118 (*bet*L^-) (0.3%) (Sheehan *et al.*, 2006). Therefore, the selection of an appropriate growth medium on a case-by-case basis is essential to maximize survival of the organisms during drying and storage.

2.2.3 Temperature and pH

Temperature is a critical factor influencing probiotic survival during drying and storage. It has been demonstrated that outlet temperature was inversely linearly related to survival of probiotic during spray drying (Gardiner *et al.*, 2000). Consistent with Santivarangkna *et al*. (2007), the outlet air temperature or the temperature at which product leaves the drying chamber is believed to be the major

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drying parameter affecting viability of spray-dried starter culture and influencing the moisture content of dried products. Many studies supported that the lower loss of viability resulted from the lower outlet temperature. Golowczyc *et al*. (2010a) demonstrated that the lowest outlet air temperature of 70 °C was associated with the lowest loss of viability of 1.0, 1.7 and 2.2 log CFU/g in *L. plantarum* CIDCA 83114, *L. kefir* CIDCA 8348 and *S. lipolytica* CIDCA 812, respectively. According to Wang *et al*. (2004), higher outlet temperature at 90 °C exhibited the higher loss of viability of probiotic (*B. longum* B6+*L. acidophilus* CCRC 14079) than that of 75 and 60 °C. This was in agreement with Hamsupo (2005) that the outlet temperature of 90 °C resulted in the lowest of survival rate of 5.2%, while the highest survival rate of *L. reuteri* KUB-AC5 after spray drying was obtained from the outlet temperature of 70 °C (82.6% survival). The extent of survival or destruction of lactic acid bacteria during spray drying not only depends on the temperature used but also depends on the heat resistance of the microorganism. The survival of *L. paracasei* NFBC 338 at outlet temperatures of 95–100 °C was 4.3% (viable cell count decreased from 1.08×10^9 to 4.8×10^7 CFU/g), while there was 0.5% survival (viable cell count decreased from 3.6×10^8 to 1.8×10^6 CFU/g) at outlet temperature of 100–105 °C. Interestingly, the survival or *L. paracasei* NFBC 338 increased when heat adaptation at 52 °C for 15 min prior to spray drying were treated. The viable cell count was 8.02×10^7 and 2.4×10^7 CFU/g after spray drying at 95–100 and 100–105 °C, respectively. However, it should be noted that too low outlet temperature could resulted in higher moisture contents affecting the stability of microorganisms during storage (Desmond *et al.*, 2002).

The pH is one of the most important factors which restrict the viability of probiotic bacteria. For example, the optimum pH for growth of *L. acidophilus* is 5.5–6.0 and the optimum growth pH for *Bifidobacteria* is between 6.5 and 7.0. Therefore, the growth of *Bifidobacteria* is retarded or inhibited below pH 5.0 or above 8.0 depended on species and strain specific (Lourens-Hattingh and Viljoen, 2001). The pH of the growth media of the probiotic culture also influences the survival during drying. Silva *et al*. (2005) demonstrated that cultures of *L. bulgaricus*

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grown under uncontrolled pH were more sensitive to spray drying than cultures grown under controlled pH 6.5. The viable cell count after spray drying of uncontrolled pH lost almost 2 log CFU/g higher than controlled pH (viability loss < 1 log CFU/g) in comparison with the number of viable cells before drying. According to this result, cell grown under controlled pH produced maximum biomass but lower resistance to drying. However, the higher resistance when cells were grown under uncontrolled pH was related with the enhanced production of heat shock proteins.

2.2.4 Protective agents

The addition of protective agents is considered as a common method for protecting cells during drying and storage. The level of cell viability after spray drying varies according to numerous factors including the strain of microorganisms and also the efficacy of the protective agents used during drying (Morgan *et al.*, 2006). The efficiency of protective agents may exert different degrees of protective effect due to their chemical characteristics and physical properties (Lian *et al.*, 2002; Peighambardoust *et al.*, 2011). Protective agents may be simple or complex components. Different sugars such as trehalose and compounds such as maltodextrin (MD), skim milk, prebiotics, starch, monosodium glutamate (MSG) and acacia gum (GA) have been employed to improve the viability of microorganisms during spray drying.

Maltodextrin is one of most extensively carrier used in drying process to prevent cellular injuries during drying, provide good oxidative stability and overcome the stickiness (Bhandari *et al.*, 1993; DePaz *et al.*, 2002; Adhikari *et al.*, 2003; Gharsallaoui *et al.*, 2007). Incorporation of maltodextrin could be beneficial due to their relative high T_g values and amorphous form are able to prevent protein unfolding during drying (DePaz *et al.*, 2002). Furthermore, maltodextrin as carrier could purposefully explore in view of its water holding and soothing properties for enzymes stabilization (Yadav *et al.*, 2009). According to Reddy *et al*. (2009), *L. plantarum* CFR 2191, *L. salivarius* CFR 2158 and *P. acidilactici* CFR 2193 spray-

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dried with 10% maltodextrin displayed more than 97% survival after spray drying at air inlet and outlet temperature of 140 and 40 °C, respectively.

Trehalose is one of the most reputed osmotic protectant against both osmotic and thermal stress (Crowe *et al.*, 1996; Crowe *et al.*, 2001; Morgan *et al.*, 2006; Santivarangkna *et al.*, 2008b; Fu and Chen, 2011). This prominent protective effect could be attributed to three factors. Firstly, trehalose molecules are able to act as an alternative to water molecules in sustaining the original conformation of the lipid bilayers of cell membrane. A schematic figure of such protective effect is shown in Figure 4. Without trehalose, the removal of water molecules causes an increase in the transition temperature of gel to liquid crystalline (the melting temperature, T_m) of the lipid bilayers. The resultant phase transition of cell membrane at room temperature upon dehydration and rehydration is detrimental to cells. Trehalose depresses the T_m and stabilizes the cell membrane in liquid crystalline state when dried, consequently limiting the potential leakage of cell membrane during such phase transition.

Secondly, trehalose has a high glass transition temperature (T_g ~ 115 °C) given it higher tendency to stay glassy compared to other non-reducing disaccharides such as sucrose. When used as a protective matrix in a dry form, the glassy state has high viscosity which slows chemical reactions such as free radical oxidation, in comparison to the crystalline state. As a result, the glassy state is considered to be capable of limiting further cellular damage that occurs at the dry state and hence providing additional protection to the cells (Crowe *et al.*, 1992).

Finally, trehalose is also able to act as an effective thermoprotectants against protein denaturation. It could stabilize cytoplasmic structures such as nucleus and ribosome upon thermal stresses (Leslie *et al.*, 1995; Patist and Zoerb, 2005; Fu and Chen, 2011). This was supported by Sunny-Roberts and Knorr, (2009) that the survival rate of *L. rhamnosus* LGG and E800 after spray drying with trehalose (air outlet 60–75 °C) was 69% (1.8×10^9 CFU/mL) and 23% (3.7×10^9 CFU/mL), respectively. Strasser *et al*. (2009) reported that the highest viability of *L. plantarum*

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IFA No. 278 cell (36.9% survival) was obtained by the addition of 32% trehalose as protectants in fluidized bed drying. The recovery of *L. delbrueckii* ssp. bulgaricus cells after drying (70 °C) is a function of the trehalose concentration in dehydration media. The optimal recovery was 1.15×10^7 MPN/mL at 0.25 M in comparison to the 1.15×10^3 MPN/mL without the addition of trehalose (Gómez Zavaglia *et al.*, 2003).

Additionally, the synergistic effects may be acquired from the combination of other protectants. Sunny-Roberts and Knorr, (2011) demonstrated that the incorporation of 12.5 g/L MSG as a component of carrier media produced an increase viability of *L. rhamnosus* GG to 80.8% which was significantly different ($P < 0.05$) from survival rates obtained when cells were dried only in trehalose media.

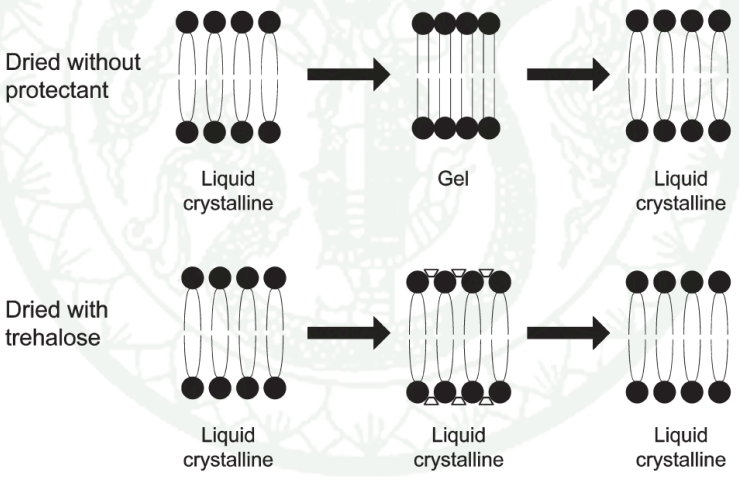

Figure 4 Schematic of the potential phase transition of cellular membrane upon dehydration and rehydration in the presence and absence of trehalose.

Source: Fu and Chen (2011)

Reconstituted skim milk (RSM) is a carrier matrix that has shown prominent effect on improving cell survival during spray drying process. It was suggested that this effective protection of RSM may be related to the lactose in RSM,

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where lactose interacts with cell membrane and helps to maintain the membrane integrity in a manner similar to those non-reducing disaccharides such as trehalose and sucrose (Corcoran *et al.*, 2004). *L. plantarum* 83114 exhibited a great survival (10 log CFU/g) after spray drying (air inlet and outlet temperature of 180 and 70 °C, respectively) in the presence of 11% skim milk. In contrast, the highest loss of viability was observed in *S. lipolytica* CIDCA 812 with the viable cell count of 7 log CFU/g even skim milk was used as protectant (Golowczyc *et al.*, 2010). The survival of *L. reuteri* KUB-AC5 reduced from 1.5×10^9 to 1.7×10^8 CFU/g after spray drying with 20% skim milk (Hamsupo *et al.*, 2005). Consistent with Ananta *et al*. (2005), *L. rhamnosus* GG remained their viability of more than 60% when 20% skim milk was used as carrier. Contrary to the reported of Chávez and Ledeboer (2007), the combination of skim milk and MD, GA or trehalose exerted the protective effect higher than that of sole skim milk. The survival rate of 100% was observed when skim milk+MD and skim milk+GA were used and survival rate of 56% was noticed in the use of skim milk+trehalose. Survival rate of only 17% was obtained when sole skim milk was used as carrier. This was in agreement with Corcoran *et al*. (2004) that use of sole RSM as carrier was reported to provide better protection on the cell viability of spray-dried *Lactobacilli* during storage than the carrier partially substituting RSM with inulin.

Moreover, protein can be used as a great coating material for encapsulation. Whey proteins used as a coating material exhibited greatly improve the survival of *L. plantarum* 299v, *L. plantarum* 800 and *L. plantarum* CIP A159 strains encapsulated in calcium alginate beads during exposure to simulated gastric fluid (SGF) condition (37 °C, 60 min). A better survival of 7.76, 6.67 and 5.81 log CFU/g (initial viable count ~ 10 log CFU/g) were observed compared to the survival in uncoated beads of 2.19, 1.89 and 1.65 log CFU/g of *L. plantarum* 299v, *L. plantarum* 800 and *L. plantarum* CIP A159, respectively (Gbassi *et al.*, 2009). A combination of skim milk and another protectant can be used to enhance the viability of spray-dried probiotics. According to Desmond *et al*. (2002), an improved survival of *L. paracasei* after spray drying and storage up to 4 weeks when using 10% arabic gum+10% skim milk instead of 20% SMP.

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Acacia gum (GA) is a polysaccharide that has a proven encapsulating and protecting capacity for sensitive materials (Krishnan *et al.*, 2005). Additionally, acacia gum has been used as an encapsulating agent by spray drying due to its good emulsifying capacity and low viscosity in aqueous solution (Gharsallaoui *et al.*, 2007; Kurozawa *et al.*, 2009). Also, Michel *et al.* (1998) revealed that acacia gum established prebiotic functionality. The addition of acacia gum enhanced the survival of *L. paracasei* NFBC 338 up to 3 log cycles (Kearney *et al.*, 2009). The survival of *L. plantarum* after air-drying enhanced from 44% in control to 79 and 66% after the addition of sorbitol and maltose, respectively (Linders *et al.*, 1997). The combination of trehalose and MSG as carrier could improve the viability of *L. rhamnosus* GG and E800 after spray drying with survival rate of 80.8% (3.1×10^9 CFU/mL) and 89.3% (1.3×10^9 CFU/mL), respectively. Comparison to trehalose without the addition of MSG, the survival rate of *L. rhamnosus* GG and E800 were 68.8% (1.8×10^9 CFU/mL) and 23.4% (3.7×10^8 CFU/mL), respectively (Sunny-Roberts and Knorr, 2009).

Prebiotics, non-digestible food ingredients, are also found to affect the host by stimulating the growth and activity of one or a limited number of bacteria in the colon in order to improve the host health. These substances have received interest as carriers of probiotic in food products with the resultant product referred to synbiotics (Corcoran *et al.*, 2006). Several researchers have studied the use of prebiotics as protectants in spray drying process. Ananta *et al*. (2005) suggested that prebiotics substances: raftilose®P95 and polydextrose (PDX) could be incorporated in spray-dried medium without any adverse impact on the viability (~40–50% survival) after spray drying. In addition, a combination of skim milk and prebiotic (PDX or inulin) in a 1:1 ratio resulted in spray-dried powders harboring numbers of *L. rhamnosus* GG up to 10^9 CFU/g (Corcoran *et al.*, 2004).

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2.2.5 Rehydration

Rehydration is considered to be a critical step in the recovery of spray-dried lactic acid bacteria. The rehydration conditions may affect the survival rate of dried microorganisms. This may lead to the rupture of cytoplasmic membrane or the leakage of cellular contents and consequently causing higher mortality (Fu and Chen, 2011). It was found that temperatures, rehydration media and the rate of rehydration play a crucial role on viability of microorganisms. Ishibashi *et al*. (1985) indicated that the optimal rehydration temperature for *S. faecalis* was 20–30 °C, while higher temperature of 30–40 °C was appropriated for *B. longum* and *L. acidophilus*. According to Mille *et al*. (2004), rehydration temperature influences the viability of *L. plantarum* and *L. bulgaricus*. There was no significant difference in viability when dried *L. plantarum* samples were rehydrated at 30 and 37 °C (1.5×10^{10} and 1.3×10^{10} CFU/g, respectively). However, the viability of *L. bulgaricus* was greatly influenced by temperature. Only 2.1×10^{9} CFU/g of viable cell count was observed when rehydration at 4 °C, whereas the viable count reached 1.5×10^{10} CFU/g at 30 or 37 °C. The viable cell count increased steadily with the temperatures increased: 3×10^{9}, 3.5×10^{9}, 5.2×10^{9} and 1.1×10^{10} CFU/g when rehydration at 10, 15, 20 and 25 °C, respectively. Wang *et al*. (2004) supported that the recovery of spray-dried cells of *S. thermophilus* and *B. longum* increased upon increasing the rehydration temperature at 35 and 50 °C. This result is in agreement with Teixeira *et al*. (1995b), the survival rate of *L. bulgaricus* increased linearly with the temperature increase between 4 and 50 °C.

Additionally, rehydration media and the rate of rehydration have been also reported as an important factor for recovery of bacteria injured during the drying process. Teixeira *et al*. (1995b) demonstrated that no significant differences ($P > 0.05$) in viability of *L. bulgaricus* were detected when skim milk, MRS broth, deionized water or phosphate buffer were used as rehydration media. Also, the slow rehydration (30 min) *L. bulgaricus* resulted in higher cell viability than that of rapid dehydration (2 min).

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3. Storage stability of probiotic

The storage conditions are critical parameters affecting the viability of dried cells. A number of factors have been shown to affect probiotic survival during storage including temperature, moisture content, relative humidity (RH) and packing condition.

3.1 Temperature

It is well established that storage temperature is a critical parameter affecting the survival of dried cells. Many studies showed that probiotic viability is inversely related to storage temperatures and this is strain dependent (Teixeira *et al.*, 1995b; Gardiner *et al.*, 2000; Krumnow *et al.*, 2009; Toledo *et al.*, 2010; Golowczyc *et al.*, 2011b). Refrigerated temperature has demonstrated to be suitable for the storage of several dried culture. At low temperature, bacterial metabolism decreases resulted in reduction rates of detrimental chemical reaction such as fatty acid oxidation and the accumulation of toxic wastes from the metabolism are likely minimized (Bucio *et al.*, 2005; Heidebach *et al.*, 2010). Golowczyc *et al*. (2010) reported that there was no significant decrease in viability during 120 days when *L. plantarum* CIDCA 83114 was kept at 6 °C. On the other hand, the higher loss of viability was observed at 20 °C. This was in agreement with Strasser *et al*. (2009) that no significant loss of viability of fluidized bed dried *E. faecium* IFA No.045 cells with and without protectants was observed throughout storage period at 4 °C.

According to Fu and Chen (2011), the mechanism of the loss of cell viability at elevated temperature is probably due to the natural degradation of life-essential macromolecules considering that *in vitro* lipids and proteins undergo oxidation and denaturation during prolonged storage period. At higher storage temperature, a greater loss of cell viability has been observed (Abe *et al.*, 2009a; Golowczyc *et al.*, 2011b). Previous finding of Castro *et al*. (1995) also demonstrated that the stability of dried microorganisms decreased during storage showing higher

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losses in viability at elevated storage temperature corresponding to a higher rate of fatty acid oxidation.

3.2 Moisture content and water activity

Moisture content is critical for optimum bacterial survival during storage. The optimum residual moisture content depends on the composition of the fluid which the bacteria are dried, the storage atmosphere and the species of bacteria (Peighambardoust *et al.*, 2011).

Water activity (a_w) represents the fraction of the water that is available to cells for metabolism and growth believed to play a major role in probiotic stability during storage (Roos, 2006). The moisture content and water activity of dried probiotic powders should be kept constant in order to achieve long-term storage stability (Chávez and Ledeboer, 2007) which should be below 5% and 0.25, respectively (Peighambardoust *et al.*, 2011). Zayed and Roos (2004) found that a residual water content of ~2.8–5.6% improved the survival rate during storage of freeze-dried *L. salivarius* UCC500. The increasing of moisture content up to 5.6% did not affect the viable cell number (~ 10^9 CFU/g) during 7 weeks of storage at room temperature. Furthermore, Ishibashi *et al.* (1985) recommended a_w values of 0.2 for maximum stability of probiotic bacteria in powders, while a_w value of 0.1 resulted in a great viability of *L. bulgaricus* during storage of spray dried powders. This was in agreement with Golowczyc *et al*. (2010) that the survival of *L. kefir* CIDCA 8321 and CIDCA 8348 was significant higher under low condition of RH (0–11%). From specific rate of degradation (*k*) of the linear regression slope, a significant increase in the decimal reduction time values at 20 °C ($D_{20,}$ weeks) were obtained in samples dried with skim milk (11% w/v) supplemented with FOS (20 g/L) and store at 11% RH. The higher rapid loss of viability at 20 °C was observed upon storage at 23% RH (the lowest D_{20} value).

Furthermore, Castro *et al*. (1995) reported that a relative humidity of about 11% is considered the best to maintain viability during storage of *L. delbrueckii*

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ssp. *bulgaricus* NCFB 1489. The viable count of the strain was 6.4, 7.5 and 3.1 log CFU/mL storage at 20 °C and equilibrated under 0, 11 and 33% RH for 132 days, respectively. Unfortunately, the totally loss of viability was observed at 59% RH. The lipid composition of the cell membrane changed with time at different RH values. The decrease in the unsaturated/saturated fatty acid index was more pronounced at high RH. Higher water activities seem to accelerate oxidation by increasing mobilization of components that are made non-reactive at low water activity by being trapped or encapsulated with in a matrix.

3.3 Protective agents

The addition of protective agents is considered to protect cells during storage. Protective agents could stabilize the cellular structures, physically reduce the environmental stress by restricting molecular movement and prevent harmful free radicals (Fu and Chen, 2011).

According to Simpson *et al*. (2005), the cell population of spray-dried *B. pyschraerophilum* LMG 21775 in acacia gum was 5.03 log CFU/g higher than in RSM (3.41 CFU/g) after storage at 25 °C for 90 days. Ananta *et al*. (2005) revealed that the protective capability of protective agents decreased in the following order: RSM > RSM+polydextrose > RSM+Raftilose®P95 when spray-dried *L. rhamnosus* GG was kept at 25 and 37 °C for 6 weeks. Similarly, the incorporation of the prebiotic substances such as polydextrose and inulin into the drying medium did not improve survival of *L. rhamnosus* GG, E800 and *L. salivarius* UCC during storage particularly at 37 °C in comparison to powder made with skim milk alone (Corcoran *et al*., 2004).

Interestingly, maltodextrin can be used to replace non fat skim milk (NFSM). Reddy *et al*. (2009) showed that the survival rate of *L. salivarius* CFR 2158 was 45% when maltodextrin were used which was higher than that of NFSM (38%) during storage at 30 °C for 60 days. Moreover, RSM supplemented with FOS provided D_{30} of *L. kefir* CIDCA 8321 higher than that of RSM and RSM+sucrose

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storage at 0% RH for 14 weeks (Golowczyc *et al.*, 2011b). Chávez and Ledeboer (2007) demonstrated that soy protein isolate (SPI)+maltodextrin exhibited the highest viable cell count (> 10^7 log CFU/g) followed by skim milk powder (SMP)+trehalose and SMP+acacia gum with the viable cell count > 10^6 CFU/g after storage at 30 °C for 90 days. However, the viable cell counts were below 1×10^6 CFU/g for SPI, SMP and SMP+MD.

The protective agents providing higher survival rate after spray drying was not necessarily related to higher survival during storage. Sunny-Roberts and Knorr (2011) demonstrated that the viable cell counts of spray-dried *L. rhamnosus* GG (with trehalose) in chocolate dairy powder was a rapid decline to undetectable value since 4th week of storage at 25 °C. Whereas, the survival rate of dried *L. rhamnosus* GG (with Tre+MSG) was maintained at a higher level (4.6×10^7 CFU/g) during storage at 25 °C over a period of 8 weeks.

Lipid oxidation can result in some physical changes in membrane functions and structure. Rapid decrease in viability tends to occurring during the early storage period, while changes in lipid composition of the cell membrane were found to increase with time (Santivarangkna *et al.*, 2008a). The increase in the proportion of saturated fatty acids causes an increase in T_m (consequently a decrease in membrane fluidity at a given temperature and an increase in membrane leakage during rehydration). Moreover, the attack of free radicals on fatty acid moieties could lower the hydrophobicity due to the introduction of hydrophilic groups and therefore weakens the hydrophobic interaction with membrane proteins which may be essential for their activity. The changes in membrane also affect some control mechanisms connected with the regulation of DNA replication because initiation of DNA synthesis and its continuation is believed to need an attachment of the DNA complex to the cytoplasmic membrane major cause of cell death may result from biological oxidations. In addition, the free radicals can also directly induce DNA damages (Santivarangkna *et al.*, 2008a). The addition of a well-known antioxidant ascorbic acid together with MSG protects *L. delbrueckii* ssp. *bulgaricus* cells only storage at 4

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°C and this protective effect is the strongest during the first two months. At a storage temperature of 20 °C, the death rate of the culture was even higher in the presence of these compounds than in the control (Teixeira *et al.*, 1995a). It was supposed that this is due to the dual properties of ascorbic acid as both an antioxidant and a prooxidant. Its antioxidant property acts as a radical scavenger, while its pro-oxidant property generates hydroxyl radicals which can attack and oxidize biological molecules. This prooxidant reaction in ascorbic acid protein mixtures seems to be dependent on concentration and temperature (Santivarangkna *et al.*, 2008a).

3.4 Glass transition temperature

The important physical property of dried powder during storage may discuss in term of the glass transition temperature (T_g). The glass transition temperature is the critical temperature at which the amorphous material changes its behavior from being glassy to being rubbery (Bhandari and Howes, 1999). This structural change is responsible for the alteration of the physicochemical properties of the products (Bhandari and Hartel, 2005; Bhandari and Adhikari, 2009). The T_g strongly depends on temperature and water content (Ananta *et al.*, 2005; Roos, 2006; Higl *et al.*, 2007). The T_g and melting point temperature (T_m) of some food materials are shown in Table 5.

Bhandari and Howes (1999) revealed that one of the factors which cause structural change of the amorphous parts of the structure is the product temperature. Additionally, Santivarangkna *et al*. (2007) suggested that storage of the dried cultures at a temperature lower than their T_g would increase the stability. Also, the addition of some compounds will raise the T_g of cultures and improve the storage viability at a given condition. Several researchers have examined the relationship between the stability of cells and T_g. Chávez and Ledeboer (2007) reported that storage stability was associated with a high $T–T_g$. At storage temperature of 30 °C, SPI+MD and SMP+GA selected as good carriers exhibited high T_g of 84.8 and 68.0 °C, respectively. Furthermore, poor stability was associated with a lower T_g for SMP (44.8 °C), SMP+Tre (49.4 °C) or SMP+MD (59.6 °C). However, a glassy state was

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not guarantee of storage stability of probiotics. For example, SPI (T_g = 95.4 °C) and SPI+sucrose (T_g = 61.6 °C) had the highest calculated $T - T_g$ but they gave provided poor survival during storage. Ananta *et al*. (2005) evaluated the contribution of glassy state on the maintenance of *L. rhamnosus* GG survival during storage. At a moisture content of 4.5% which was assumed as the residual moisture content achievable by spray drying at an air outlet temperature of 80 °C, the T_g values of RSM, RSM:Raftilose®P95 and RSM:polydextrose were 50.6, 49.5 and 44.5 °C, while the specific rate of degradation (*s*, week^{-1}) at 25°C was 0.087, 0.189 and 0.089, respectively. From the results, although all carriers were in the glassy state, different degrees in their capacity to confer protection on *L. rhamnosus* GG were observed.

Table 5 Values of T_g and T_m of some anhydrous component and foods.

Food materials	T_g (°C)	T_m (°C)
Fructose	16	108
Glucose	36	143
Galactose	38	165
Sucrose	67	192
Maltose	92	165
Lactose	101	223
Lactic acid	–60	17
Maltodextrin		
DE 36 (MW 500)	100	
DE 25 (MW 720)	121	
DE 20 (MW 900)	141	
DE 10 (MW 1800)	160	
DE 5 (MW 3600)	188	
Starch	243	

Source: Modified from Bhandari and Hartel (2005)

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3.5 Packaging

The packaging material has also been reported to affect bacterial survival during storage. The permeation of oxygen through packaging during storage affected the viability of microorganisms (Ishibashi and Shimamura, 1993; Wang *et al.*, 2004; Otero *et al.*, 2007). The accumulation of free radicals such as oxygen species within cells results in irreversible damaging processes occurring within the cell (Bozoglu *et al.*, 1987). Ishibashi and Shimamura (1993) indicated that the higher the oxygen permeability of package, the lower the viability is. In addition, material with high water vapor permeability also results in poor survival rate (Corcoran *et al.*, 2006). Farnworth and Champagne (2010) suggested that the moisture in the air could be able to increase a_w of the culture powder during storage.

In order to prevent/reduce such an oxidative phenomenon, dried powders should be stored under vacuum or under controlled water activity (Otero *et al.*, 2007). Chávez and Ledeboer (2007) also supported that packages under vacuum or nitrogen replacement are suitable for storing anaerobic probiotic such as *Bifidobacterium*. Vacuum storage was shown to be better than nitrogen and air. Otero *et al*. (2007) reported that the viability of dried *L. gasseri* CRL1412 in RSM kept in sealed glass ampoules (7.12 log CFU/g) was lower than that of glycogelatine capsules (8.97 log CFU/g) after storage at 2–8 °C for 270 days. Moreover, Wang *et al*. (2004) demonstrated that the survival of *B. longum* B6 in the spray-dried fermented soymilk held in the laminated pouch was 57.7% (6.83 log CFU/g) higher than that of glass bottle (57% survival) and PET bottle (33.6% survival). These were in the same trend as spray-dried *S. thermophilus* CCRC 14085. The survival of the strain held in the laminated pouch reduced from an initial population of 7.51 to 6.97 log CFU/g (29.5% survival). Whereas, lower survival rates of 18.8 (6.78 log CFU/g) and 15.7% (6.70 log CFU/g) was obtained in glass bottle and PET bottle kept at 4 °C for 4 months, respectively.

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The stability of microorganisms in food products during storage has a great impact on the product quality. In practical, especially when the actual storage time is long, an accelerated storage testing is used in order to develop a mathematical model for predicting the microorganism stability considered the shorter time and process (Mizrahi, 2000; Abe *et al.*, 2009a). The Arrhenius equation based on thermodynamic considerations has had notable success in describing the temperature dependence of many chemical reactions related to the shelf-life of foods (Labuza and Fu, 1993; Mizrahi, 2000).

The accelerated storage test based on Arrhenius equation has been used to extrapolate the shelf-life of microorganisms. Several studies were demonstrated for their predictive model of various microorganisms (Table 6). From this review, the accelerated storage testing is a potential extrapolation tool for estimation of the bacterial shelf-life. However, the real-time storage testing must be done in parallel to confirm the shelf-life prediction (Wirunpan, 2011).

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Table 6 Prediction model of various microorganisms.

Microorganisms/ Process condition	Prediction model	Descriptions	References
B. longum ssp. *longum* BB536/ -lyophilized -storage at various a_w and temperatures	$\log \frac{N_t}{N_0} = \frac{-\exp(k'x + C)}{t}$ This equation was derived to estimate the survival rate of *Bifidobacteria* powder under each a_w and temperature condition.	There were no great difference between the theoretical and the actual survival rates for various conditions.	Abe *et al*. (2009)
B. subtilis ATCC 6051/ conventional drying at 40–90 °C	$\log A = \log(\frac{ekT}{h}) + \frac{\Delta S}{2.303R}$ The incorporation of Arrhenius and Eyring equation	Predicted and experimental results are in a good agreement.	Sorokulova *et al*. (2008)
L. acidophilus BCRC 10695/ free and immobilized cells-fermented banana media were subjected to freeze-drier and storage at 4 and 25 °C	The results were shown in term of predicted *k* values (h^{-1}) of free and immobilized cell-fermented banana after freeze-dried.	The predicted and experimental results showed no significant difference by *t*-test at 95% level confidence.	Tsen *et al*. (2007)

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Table 6 (Continued)

Microorganisms/ Process condition	Prediction model	Description	References
L. reuteri KUB-AC5/ spray drying at air inlet of 170 °C and air outlet of 85 °C	$\log N = \log N_0 - 1.286 \times 10^{-3} t$ (at 4 °C) $\log N = \log N_0 - 0.031t$ (at 30 °C)	Comparison of prediction and experimental survival rates did not show any significant differences for 4 months of storage.	Hamsupo *et al*. (2005)
L. brevis ATTC 8287/ freeze drying	$k = 1.97 \times 10^{-4}$ h^{-1} (at 4 °C)	Comparison of the estimated and experimental measured survival rate was fit during storage for 50 days and a difference of 30% was detected after 137 days storage.	Desmond *et al*. (1998)

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MATERIALS AND METHODS

Materials

1. Microorganisms

Lactic acid bacteria isolated from fermented vegetables were preserved in de Man, Rogosa, Sharpe (MRS) broth with 20% (v/v) glycerol content at –20 °C. *Escherichia coli* O157:H7 DMST 12743 and *Salmonella* Typhimurium ATCC 13311 were purchased from the Department of Medical Science, Ministry of Public Health, Thailand. The indicator strains were grown in tryptic soy broth (TSB) supplemented with 0.6% yeast extract (YE) at 37 °C. All strains were subcultured twice and incubated at 37 °C for 24 h under microaerobic-static condition and then used as inoculum.

2. Culture media

2.1 de Man, Rogosa, Sharpe (MRS) broth (Merck, Darmstadt, Germany)

2.2 Tryptic soy broth (TSB) (Difco Laboratories, Detroit, MI, USA)

3. Chemicals

3.1 Acacia gum (Commercial grade)

3.2 Agar powder (Merck, Darmstadt, Germany)

3.3 Ascorbic acid ($C_6H_8O_6$; Unilab, NSW, Australia)

3.4 Bile salt (Oxoid, Basingstoke, Hampshire, UK)

3.5 Calcium carbonate ($CaCO_3$; precipitated, reagent grade, Scharlau Chemie, S.A.)

3.6 3, 5-dinitrosalicylic acid ($C_7H_4N_2O_7$; Fluga, China)

3.7 Dipotassium hydrogen phosphate (K_2HPO_4; Merck, Germany)

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3.8 95% Ethyl alcohol (C_2H_5OH; Solvent solution Grade A; Commercial grade)

3.9 Soy protein isolate (73.5%) mixed with milk protein concentrate (24%) (Nutrilite™, Amway, USA)

3.10 Fibersol®-2 (Indigestible dextrin; Matsutani, Japan)

3.11 D-glucose ($C_6H_{12}O_6$; Univar, Ajak, NSW, Australia)

3.12 Glycerol ($C_3H_8O_3$; Univar, Ajak, NSW, Australia)

3.13 Isomalt ($C_{12}H_{24}O_{11}$; Beneo, Germany)

3.14 Lactic acid ($C_3H_6O_3$; Sigma, Japan)

3.15 Lithium chloride (LiCl; Sigma-Aldrich, Germany)

3.16 Magnesium chloride ($MgCl_2$; Scharlau, Spain)

3.17 Maltodextrin DE10–12 ($C_6H_{12}O_6$; Du Zhi Xue, China)

3.18 Palatinose ($C_{12}H_{22}O_{11}$; Beneo, Germany)

3.19 Pancreatin USP (P-1500, Sigma, Basingstoke, Hampshire, UK)

3.20 Paper discs containing the antibiotics (BD BBL™, Becton Dickinson, MD, USA)

3.21 Pepsin from porcine gastric mucosa (P-7000, 1:10,000, ICN, Sigma, Basingstoke, Hampshire, UK)

3.22 Phenol (C_6H_5OH; BDH Prolabo, EC)

3.23 Phosphorus pentoxide (P_2O_5; saturated salt solution, ACROS Organic, USA)

3.24 Potassium acetate (CH_3COOK; Unilab, Australia)

3.25 Sodium Chloride (NaCl; Univar, Ajak, NSW, Australia)

3.26 Sodium hydroxide (NaOH; Merck, Germany)

3.27 Sulfuric acid (H_2SO_4; Lab-Scan, Ireland)

3.28 Trehalose ($C_{12}H_{22}O_{11}$;Hayashibara, Japan)

3.29 Toluene (C_7H_8; Panreac Quimica SAU, Barcelona, Espana)

3.30 Xylene (C_8H_{10}; Panreac Quimica SAU, Barcelona, Espana)

3.31 Yeast extracts (Difco Laboratory, Detroit, MT, U.S.A.)

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4. Equipments

4.1 AquaLab 3TE Water Activity meter (Decagon Devices, Pullman, WA, USA.)

4.2 Autoclave (Tomy, Model ES-315, Japan)

4.3 Balance 2 digits (Sartorius, Model ED32023, Germany)

4.4 Balance 4 digits (Ohaus, NJ, USA.)

4.5 Centrifuge (Hermle, Germany)

4.6 Differential scanning calorimeter (DSC1 Star^{e} system, Mettler Toledo, Schwerzenbach, Switzerland)

4.7 Digital refractometer (Leica Microsystem, Inc., New York, USA)

4.8 DSC aluminium crucible pan (Mettler Toledo, 40 µL; ME-51119870, Greifensee, Switzerland) and lid (Mettler Toledo, ME-51119871, Greifensee, Switzerland)

4.9 Hot air oven (Memmert, Model UNE 200, Germany)

4.10 Incubator (Memmert, Model UNE 200, Germany)

4.11 Laboratory scale spray dryer (Büchi B-290, Flawil, Switzerland)

4.12 Laminar flow (NuAire, NU440, USA)

4.13 Light microscope (ZEISS, Axiostar plus with AxioCam, ERc5S, Germany)

4.14 Membrance filter 0.22 µm (Minisarts®, satorius stedim, Goettingen, Germany)

4.15 Orbital incubator shaker (n-Biotek, NB205, Korea)

4.16 Pilot scale spray dryer (GEA Niro A/S, Denmark)

4.17 pH meter (Mettler Toledo, FiveEasy, Switzerland)

4.18 Plastic container (TechnoPlas, Australia)

4.19 Scanning electron microscope (JSM 5600 LV, JEOL Ltd, Tokyo, Japan)

4.20 UV-Vis spectrophotometer (Shimadzu Co., Kyoto, Japan)

4.21 Vacuum oven (Thermoline Scientific, Australia)

4.22 Vortex mixer (Genie, Scientific Industries, USA)

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Methods

1. Isolation of lactic acid bacteria with high acid production

The isolation of lactic acid bacteria from fermented vegetables was carried out by pour plate method using MRS agar containing $CaCO_3$ as a preliminary screening medium. The preparation of fermented vegetables was shown in Appendix A. To isolate the lactic acid bacteria, 25 g of fermented vegetables samples were taken aseptically and transferred to 225 mL of 1% peptone water and vigorously shaken for 120 s using the Stomacher. Appropriate decimal dilutions were prepared in 0.85% NaCl and poured into a sterile petri dish on MRS agar containing 0.5% (w/v) $CaCO_3$. Then, all plates were incubated at 37 °C for 24 h. A number of bacterial colonies exhibited clear zone on the plates were randomly selected and individually picked and re-streaked on MRS agar plates to obtain pure culture. All strains were preliminarily tested for their growth under different conditions (pH, NaCl and temperatures), lactic acid production and fermentative type. The strains with high acid production (13 strains) were selected and identified using biochemical method. All 13 strains identified as *L. plantarum* have been deposited in the Microbiological Resources Center (MIRCEN), Institute of Scientific and Technological Research (TISTR), Thailand and used for further studies.

2. Determination of probiotic properties

2.1 Autoaggregation assay

Autoaggregation assays were performed according to Del Re *et al.* (2000). Overnight cultures were harvested by centrifugation at 5,000 *g* for 15 min. The cell pellets were washed twice and resuspended in sterile phosphate buffer saline (PBS) to give viable cell counts of approximately 1×10^8 CFU/mL. The cell suspensions (4 mL) were vortexed for 10 s. During incubation at room temperature for 5 h, 0.1 mL of the upper suspensions was transferred to another tube containing 3.9 mL of phosphate buffer saline (PBS) and the optical density (OD_t) was measured at 600 nm

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every 1 h. The autoaggregation percentage was expressed as $\left[1-\left(\frac{OD_t}{OD_0}\right)\right]\times 100$, where OD_t represents the optical density at time t = 1, 2, 3, 4 and 5 h and OD_0 is the optical density at t = 0.

2.2 Coaggregation assay

Coaggregation between *L. plantarum* and *E. coli* O157:H7 DMST 12743 or *S.* Typhimurium ATCC 13311 was investigated. The cell suspensions were prepared in the same manner as described in the autoaggregation assay. Equal volumes (2 mL) of *L. plantarum* and pathogen cell suspension were mixed together by vortexing for 10 s. Control tubes were set up at the same time, containing 4 mL of each bacterial suspension on its own. The optical density at 600 nm of the suspensions was measured after 5 h of incubation at room temperature. Samples were taken using the same procedure as in the autoaggregation assay. The percentage of coaggregation was calculated using as the following (Handley *et al.*, 1987):

$$\text{Coaggregation (\%)} = \frac{\left(\frac{A_x + A_y}{2}\right) - A_{(x+y)}}{\left(\frac{A_x + A_y}{2}\right)} \times 100$$

where x and y represent *L. plantarum* and the pathogen, respectively and (x+y) represents the mixture of *L. plantarum* and each pathogen.

2.3 Cell surface hydrophobicity assay

Cell surface hydrophobicity was determined by the method of Kos *et al.* (2003) with minor modifications. Overnight cultures of *L. plantarum* were harvested by centrifugation at 5,000 *g* for 15 min. The cell pellets were washed twice and resuspended in sterile 0.85% NaCl solution to give an absorbance of 0.5 at 600 nm (A_0). To test tubes containing 3 mL of washed cells, 1 mL of toluene or xylene was added. The mixtures were vortexed for 90 s. After incubation at room temperature for 15 min, the aqueous phase was removed and its absorbance at 600 nm (A_1) was then

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measured. The percentage of cell surface hydrophobicity was calculated as $\left[1-\left(\frac{A_1}{A_0}\right)\right] \times 100$.

2.4 Antibiotic resistance

The antibiotic sensitivity of *L. plantarum* was determined by the Bauer-Kirby method (Bauer *et al.*, 1966). The optical density at 600 nm of the overnight culture was adjusted to 0.08–0.1 (equivalent to 1–2 × 10^8 CFU/mL). The inocula were spread evenly over the entire surface of the MRS agar plates. Subsequently, paper discs containing the antibiotics were laid on the plates. After incubation at 37 °C for 24 h, the inhibition zones were measured inclusive of the diameter of the discs. Antibiotics tested were classified into 4 groups: (i) inhibitors of cell wall synthesis: penicillin (P), cepfoxitin (FOX), vancomycin (VA), oxacillin (OX), bacitracin (B), ampicillin (AM); (ii) inhibitors of protein synthesis: kanamycin (K), streptomycin (S), tetracycline (TE), chloramphenicol (C), erythromycin (E), clindamycin (CC) (iii) inhibitors of nucleic acid synthesis: ciprofloxacin (Cip), nalidixic acid (NA), rifampin (RA), trimethroprim (TMP); (iv) inhibitors of cytoplasmic membrane function: polymyxin B (PB). Results were expressed as sensitive, S (diameter ≥ 21 mm); intermediate sensitive, I (diameter 16–20 mm) and resistant, R (diameter ≤ 15 mm) (Vlková *et al.*, 2006).

2.5 Antimicrobial activity

Overnight culture (200 µL) of *E. coli* O157:H7 DMST 12743 or *S.* Typhimurium ATCC 13311was mixed with 20 mL of melt TSAYE (approximately 1 × 10^6 CFU/mL) and poured onto sterile Petri-dishes. Wells (7 mm-diameter) were punched out of the solid agar with a sterile cork borer. Overnight culture of *L. plantarum* (50 µL) was introduced into the wells and the plates were incubated at 37 °C for 24 h (Domrongpokkaphan and Wanchaitanawong, 2006). The diameter of inhibition zones was measured. Each experiment was repeated in triplicate.

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Cell-free supernatants (CFS) of all overnight cultures were evaluated for antimicrobial activity by the spot-on-lawn method as described by Alemu *et al.* (2002). Each CFS was also adjusted to pH 5.0 and 6.0 with 5 M NaOH to eliminate the inhibitory effect of organic acid and filter-sterilized with disposable bacterial filters 0.22 μm. A TSAYE plate (1.5% agar) was overlaid with 5 mL of soft TSAYE (0.75% agar) containing 10 μL of indicator strain (*E. coli* O157:H7 DMST 12743 or *S.* Typhimurium ATCC 13311) (approximately 1×10^6 CFU/mL). Serially diluted CFS (10 μL) was spotted onto the indicator plates. The plates were then incubated at 37 °C for 6 h. The inhibition zone was revealed by the formation of a clear zone in the indicator bacterial lawn. Antimicrobial activity was expressed in arbitrary units (AU) per milliliter of the original cultures. An arbitrary unit was defined as the reciprocal of the highest dilution which produces a clear zone of growth inhibition of the indicator strain calculated as $\frac{(D \times 1000)}{10}$, where D denotes the dilution factor.

2.6 Simulated gastrointestinal tract tolerance

2.6.1 Preparation of gastric and small intestinal juice

Simulated gastric juice was prepared by means of suspension of pepsin in sterile 0.5% NaCl to a final concentration of 3 g/L and adjusted to pH 2.0 with concentrated HCl (Michida *et al.*, 2006). Simulated small intestinal juice was prepared by suspension of pancreatin USP in a sterile 0.5% NaCl to a final concentration of 1 g/L then added with 0.45% bile salt and adjusted to pH 8.0 with sterile 0.1 M NaOH (Huang and Adam, 2004).

2.6.2 Preparation of washed cell suspension

L. plantarum was grown in MRS broth at 37 °C for 20 h. Cell culture of each test strain was centrifuged at 5,000 *g* for 10 min. After washing twice with sterile saline, the cell pellet was resuspended in the same solution. The viable

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cell count of the washed cell suspension was determined prior to assay of transit tolerance.

2.6.3 Determination of gastrointestinal tract tolerance

An aliquot of each washed cell suspension (0.2 mL) was transferred to a sterile tube, mixed with sterile 0.5% NaCl (0.3 mL) and finally blended with 1.0 mL of simulated gastric juice (pH 2.0) or small intestinal juice (pH 8.0) in the presence of 0.45% bile salt. In the simulated gastric juice tolerance determination, viable cell counts were measured after 30, 60, 90 and 180 min. In the simulated small intestinal juice tolerance determination, viable cell counts were measured after 240 min.

2.7 Heat tolerance

Heat tolerance of *L. plantarum* was determined according to Ding and Shah (2007). Overnight cultures of *L. plantarum* were incubated at 65 °C. The cell viability was monitored at 0, 30 and 60 min.

3. Effect of cereal flour on gastrointestinal tract tolerance

3.1 Preparation of cereal flour and chemical analysis

Soybean, sesame and Job's Tears grains were ground and separated with a sieve size of 0.5 mm. The resulting powder (5 g) was mixed with distilled water (45 mL) and sterilized at 121 °C for 15 min before used as 10% (w/v) cereal suspension.

Total sugar concentration was determined by the phenol-sulphuric acid method (Dubois *et al.*, 1956) and the sample analysis was conducted based on a calibration curve (R^2 = 0.993) by the application of an array of glucose standard solution (400 mg/L). Reducing sugar concentration was determined by the 3, 5–dinitrosalicylic acid method (Miller, 1959) and the sample analysis was conducted

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on the basis of a calibration curve (R^2 = 0.993) employing an array of glucose standard solutions (1 g/L). The buffering capacity of samples was determined by titrating 100 mL of the medium with HCl (1 N). The value was expressed as the amount of HCl (mmol) required dropping 1 pH unit per unit volume (L).

3.2 Tolerance of *L. plantarum* in simulated gastrointestinal tract in the present of cereal flour

The tolerance of washed cell suspensions of *L. plantarum* strains to simulated gastric and small intestinal juices was determined as described above; with the exception that 0.3 mL of 10% (w/v) cereal suspension replaced the sterile saline addition. Survival of *L. plantarum* was determined by the standard plate count method using MRS agar.

3.3 Survival of *L. plantarum* after sequential incubation in simulated gastric and small intestinal juices

After exposure to simulated gastric juice (pH 2.0, 180 min), the cell suspension were centrifuged at 5,000 *g* for 10 min and subsequently resuspended in simulated small intestinal juice (pH 8.0 with 0.45% bile salt) (Valerio *et al.*, 2006). The suspensions were then incubated at 37 °C for 240 min. The samples for total viable cell counts were taken at 60, 90 and 180 min during exposure to simulated gastric juice and at 60, 120 and 240 min during exposure to simulated small intestinal juice. The same procedure was used with addition of the cereal suspension. Survival of *L. plantarum* was determined by the standard plate count method using MRS agar.

4. Fermentation of cereal extracts

Fermented cereal extracts were prepared according to the procedures described by Wang *et al*. (2002). Cereals (soybean and Job's Tears) were washed and soaked in distilled water. The soaked cereals were mixed with distilled water (bean:water = 1:10 w/v). After decanting the soaking water, the soaked cereals were

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mixed with distilled water, added with different concentrations of sesame (1 and 2% w/v) and then comminuted in a blender for 3 min (Hou *et al.*, 2000). The resultant slurry was filtered through double-layered cheesecloth 2 times to yield cereal extracts. Each of cereal extracts was dispensed into containers, added with different concentration of glucose and sterilized by heating for 15 min at 121 °C. Sterilized cereal extracts were inoculated with overnight culture of 1% (v/v) of *L. plantarum*. Fermentation was carried out at 37 °C for 24 h. Viable cell counts were determined by the standard plate count method with MRS medium at 37 °C. pH was measure with a pH meter. Soluble calcium of fermented cereal extracts was also evaluated by atomic spectrophotometry.

5. Spray drying of *L. plantarum*

5.1 Preparation of spray-dried powders

Firstly, the fermentation of selected *L. plantarum* in cereal extracts mixed with 1% sesame was carried out in accordance with the method section 4 above. After 24 h-fermentation, the viability of *L. plantarum* was determined by standard plated count and pH of fermented cereal extracts was carried out using pH meter.

Prior to spray drying, the culture of *L. plantarum* was mixed with 20% (w/v) maltodextrin DE10 (MD) and 5% (w/v) of each of protective agent used as follows: trehalose (Tre), the combination of soy protein isolate (73.5%) and milk protein concentrate (24%) (Prot), fibersol-2 (Fib), palatinose (Pal), isomalt (Iso), acacia gum (GA) or 0.1% ascorbic acid (Asc). The culture suspension was fed to a pilot-scale spray drier at a constant air inlet and outlet temperatures of 130 and 70 °C, respectively. The spray-dried powders were collected in a single cyclone separator and analyzed for viable cell counts. Moisture content was determined according to the GEA Niro analytical method A 1 a (IDF Standard 026, 2004).

For the determination of physical properties of spray-dried powders, the powders were prepared in laboratory scale spray drier. The protective agents as

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follows: Tre, Prot, Fib and GA were used. The suspension was fed to a laboratory scale spray drier at a constant air inlet and outlet temperatures of 120 and 60 °C, respectively. The spray-dried powders were collected in a single cyclone separator and analyzed for viable cell counts. Additionally, in case of no carrier added treatment, fermented concentrated cereal extracts (total solid was ~ 7 °Brix) were directly subjected to laboratory scale spray dryer. Moisture content of all samples after spray drying were analyzed according to AOAC method 925.45 (AOAC, 2005) and a_w was determined by using AquaLab 3 water activity meter.

5.2 Determination of antimicrobial activity and simulated gastrointestinal tract tolerance of spray-dried powders

The spray-dried samples (1% w/v) were inoculated into MRS broth and cultured 37 °C for 24 h and the inhibitory activity against *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311 were evaluated according to the method in section 2.5. For the determination of the tolerance to simulated gastric and small intestinal juices, spray-dried powder (1 g) was resuspended in 9 mL of 0.85% NaCl and evaluated according to the method in section 2.6.3.

6. Storage stability of probiotics in spray-dried powders

6.1 Effect of storage temperatures and protective agents on the viability of spray-dried *L. plantarum*

The spray-dried samples stored in sealed aluminium foil bags (7.5 × 12 cm) and kept at 4 and 25 °C were evaluated for their survival until no viable cell number was detected. The specific rate of degradation (k, day^{-1}) of spray-dried *L. plantarum* was calculated as a first-order reaction from $k = {}^{1}/_{t} \times (\log N_0 - \log N)$, where N refers to the bacterial count at a particular storage period (CFU/g), N_0 represents the bacterial count at the beginning of the storage (CFU/g) and t is the

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storage time (Desmond *et al.*, 1998; Ananta *et al.*, 2005; Hamsupo *et al.*, 2005; Tsen *et al.*, 2007; Foerst *et al.*, 2011).

6.2 Effect of relative humidity on the viability of spray-dried *L. plantarum* kept under different storage temperatures

Each sample of spray-dried powders (5 g) was placed in open plastic container inside a desiccator equilibrated over saturated salt solution LiCl, CH_3COOK, and $MgCl_2$ providing relative vapor pressure values of 11, 23 and 33%, respectively. All desiccators were then held at 4, 25 and 40 °C. The spray-dried powder samples at different relative humidity and storage temperatures were carried out for their residue viability at interval time as follows: samples were taken out at 0, 15, 45, 90, 135 and 180 days at 4 °C; samples were taken out at 0, 15, and 45 days at 25 °C and samples were taken out at 0, 7, 15 and 30 days at 40 °C.

6.3 Effect of relative humidity and temperatures on physical property of spray-dried powders

All samples of spray-dried powders kept under various relative humidity (11, 23 and 33% RH) and storage temperatures (4, 25 and 40 °C) were analyzed for their physical property in term of glass transition temperature (T_g) using a differential scanning calorimeter (DSC).

7. Accelerated storage test

The spray-dried samples were incubated at 37, 45, 60 and 80 °C and collected at a constant interval of time to measure the residue viable cell counts to calculate specific rate of degradation.

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8. Analytical procedure

8.1 Viable cell counts

Viable cell counts were determined by the standard plate count method on MRS agar plate. The plates were incubated at 37 °C for 24 h. The viable cell counts were expressed as $\log_{10}$ value/mL. The percentage of cell survival was defined as follows: $\text{survival rate } (\%) = \left(\frac{\log N}{\log N_0}\right) \times 100$, where N represents the number of viable cells (CFU/mL) after exposure and N_0 denotes the initial viable cell count (CFU/mL) prior to exposure (Bao *et al*., 2010).

8.2 Glass transition temperature (T_g)

A differential Scanning Calorimeter (DSC) was used to determine the T_g of all spray-dried powders. The calorimeter was calibrated according to the instruction provided by Mettler Toledo user manual by checking temperature and heat flow of indium as standard. Sample of 5–10 mg was weighted into a 40 µL DSC aluminum crucible pan and press sealed with a lid using a DSC sample press. Thermal scanning was carried out using sealed empty crucible as a reference in three steps: (1) heating from –10 °C to 180 °C at 10 °C/min; (2) cooling from 180 °C to –10 °C at 10 °C/min; and (3) heating from –10 °C to 180 °C at 10 °C/min. The T_g values were analyzed from the first heat scanned curve using the STAR^e software at onset, midpoint and endset points. The analysis was carried out in duplicate.

8.3 Scanning electron microscopy (SEM)

The spray-dried powders, rehydrated spray-dried samples and *L. plantarum* cell after exposure to simulated gastric juice (pH 2.0) with soybean, sesame and Job's Tears flour were monitored by scanning electron microscope. For spray-dried powders, the samples were coated with sublimated 1% osmium tetroxide for 3 h and kept in a desiccator for a week.

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In case of rehydrated spray-dried samples and *L. plantarum* cell after exposure to simulated gastric juice with cereal flour, the samples were filtered through 0.2 μm pore size sterile filter. Then, the samples on a sterile filter were first fixed with a 2.5 % glutaraldehyde in sodium phosphate buffer pH 7.2 for 12 h. After washing three times with phosphate buffer, the samples were fixed with 1% osmium tetroxide for 1 h followed by washing with distilled water three times. The samples were then dehydrated through graded series of ethanol soaks (30, 50, 70, 90 and 100 % ethanol, using 100 % ethanol three times) and drying with liquid carbon dioxide.

Finally, all samples mentioned above were attached to a brass stub with double-sided adhesive tape, coated with a layer of gold and determined with the application of a scanning electron microscope.

8.4 Soluble calcium

Soluble calcium of fermented cereal extracts was determined according to Tang *et al*. (2007). Aliquots were centrifuged at 10,000 *g* for 30 min. After centrifugation, samples were filtered through a 0.22 μm membrane before measurement of soluble calcium content by atomic absorption spectrophotometry.

8.5 Moisture content

Moisture content of spray-dried samples was determined according to the GEA Niro analytical method A 1 a. The moisture content of spray-dried powders was determined by oven drying at 102 °C (IDF Standard 026, 2004) or by vacuum oven at 70 °C according to AOAC Official method 925.45 (AOAC, 2005).

8.6 Lactic acid content

The supernatant of culture (2 mL) mixed with distilled water (18 mL) was titrated with 0.1 M NaOH. Phenolphthalein (1 mL) was used as an indicator. Each

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milliliter of 1 N NaOH is equivalent to 90.08 mg of lactic acid. The titratable acid was then calculated according to AOAC method (1990):

$$\% \text{ Titratable acidity} = \frac{\text{Volume of NaOH used} \times \text{Normality of NaOH solution} \times 90.08 \times 100}{\text{Volume of sample used} \times 1000}$$

The lactic acid content was also determined using High Performance Liquid Chromatography (HPLC) equipped with a Lichrocart C18 column (250 × 4 mm). The analysis was achieved by using 0.025 M H_3PO_4 pH 3.0 as a mobile phase with flow rate of 1.6 mL/min at room temperature and RI detector was employed.

8.7 Reducing sugar

Reducing sugars were determined by the 3, 5-dinitrosalicylic acid (DNS) colorimetric method, with glucose as the standard (Miller, 1959). DNS reagent (1 mL) was added to 1 mL of sample supernatant. The solution was boiled in a boiling water bath for 5 min and left it cool in ice bath. Then, 10 mL of distilled water was added. The absorbance at 540 nm was measured interpolating the value obtained with calculated values for glucose solutions of known concentration. The blanks were prepared by substituting sample solution for distilled water.

8.8 Total sugars

A modified phenol-sulfuric acid method was used to determine total sugars present in the samples (Dubois *et al.*, 1956). 1 mL of 5% phenol was added to 0.1 mL of sample. Then, 1 mL of concentrated sulphuric acid was added and the mixture was kept in a boiling water bath for 10 min. After cooling to room temperature, the absorbance at 490 nm was measured. The amount of sugars was then determined by reference to a standard curve prepared with glucose. The blanks were prepared by substituting sample solution for distilled water.

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8.9 Statistical analysis

Each result was expressed as the mean ± S.D. of three determinations. The data were assessed using analysis of variance (ANOVA) with a level of significance at $P < 0.05$. Significant divergences among mean values were determined with Duncan's multiple range tests. All statistical analyses were performed using SPSS Software, version 12 (SPSS, now a part of IBM Corp.; White Plains, NY, USA).

9. Place and duration

9.1 Department of Biotechnology, Faculty of Agro-industry, Kasetsart University, Thailand

9.2 School of Agriculture and Food Sciences, Faculty of Natural Resources, Agriculture and Veterinary Science, The University of Queensland, St. Lucia, Brisbane, Queensland, Australia

All experiments were carried out from April 2007 to May 2011.

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RESULTS AND DISCUSSION

1. Isolation of lactic acid bacteria from fermented vegetables

A total of 82 isolates was preliminarily screened from fermented vegetables for their ability to produce acid on MRS agar supplemented with $CaCO_3$ used as an indicator for acid-producing strains (Hwanhlem *et al.*, 2011). All isolates exhibited homofermentative characteristic with lactic acid production ranging from 1.92 to 2.33% (Appendix B). Their optimum growth conditions were 37 °C and pH 4–7. Also, it was observed that the addition of salt resulted in decreased growth of test strains and all strains could not grow at 10% NaCl.

With high acid production, 13 isolates (PKWA6-1, PKWA7-1, PKWB6-3, PKWB6-12, PKWB7-1, PKWB7-2, PKWC6-1, PKWC6-9, PKWC7-1, PKWC8-1, PKWD6-10, PKWD7-1 and PKWD7-2) were selected and characterized by physiological and biochemical tests. All selected isolates were Gram-positive, facultative anaerobic, catalase-, urease- and oxidase-negative, non-motile and non-spore forming bacteria. Moreover, all isolated fermented dextrose, fructose, lactose, maltose, mannose, mannitol, salicin, xylose and arabinose. They could not hydrolyze starch. No lipase and lecithinase production was observed. From the results mentioned above, all isolates were identified as *L. plantarum* (Appendix C). At present, they have been deposited in the Microbiological Resources Center (MIRCEN), Institute of Scientific and Technological Research (TISTR), Thailand and obtained the TISTR number as TISTR 2070–2082 (Appendix D). Additionally, they were further evaluated on probiotic properties including autoaggregation ability, cell surface hydrophobicity, coaggregation ability, antibiotic resistance, antimicrobial activity, tolerance to gastrointestinal tract conditions and heat tolerance.

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2. Probiotic properties

2.1 Aggregation and cell surface hydrophobicity

Aggregation and cell surface hydrophobicity were used to preliminary screen for probiotic properties which have been proposed as an indirect method for evaluation the adhesion ability of bacteria.

As shown in Table 7, all *L. plantarum* strains exhibited a strong autoaggregation of 63.80–86.97% after 5 h incubation. *L. plantarum* TISTR 2072 showed the highest autoaggregation ability, while *L. plantarum* TISTR 2071 displayed the lowest autoaggregation percentage. For *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311, it was observed that the autoaggregation ability was 73.48 and 77.22%, respectively. Collado *et al*. (2007) suggested that autoaggregation correlates with adhesion which is a prerequisite for colonization and infection of the gastrointestinal tract by many pathogens. Also, these results were in agreement with Del Re *et al*. (2000) that *B. longum* B7, B8, B10, B1604 and B2352 showed a high autoaggregation percentage (≥ 80%). In addition, *B. bifidum* I6 and *B. longum* I3 isolated from human feces also exhibited autoaggregation of 73.8 and 73.1%, respectively (Vlková *et al.*, 2008).

Of 13 strains, *L. plantarum* TISTR 2072, TISTR 2073, TISTR 2075, TISTR 2079, TISTR 2081 and TISTR 2082 showed coaggregation ability with both *E. coli* O157:H7 DMST 12743 (3.67–18.75%) (Figure 5) and *S.* Typhimurium ATCC 13311 (3.85–12.16%).While *L. plantarum* TISTR 2078 showed only coaggregation ability with *E. coli* O157:H7 DMST 12743 (8.11%). The coaggregation is thought to be linked to the ability to interact closely with undesirable bacteria representing competitive exclusion of the test strains against enteric pathogens (Taheri *et al.*, 2009b). This result was in close agreement with the finding of Kos *et al*. (2003) that the coaggregation percentage of *L. acidophilus* M92 and pathogens were 15.11% (with *E. coli* 3014) and 15.70% (with *S.* Typhimurium), respectively. Hence, it could

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be indicated that the coaggregation properties were strain-specific (Kos *et al.*, 2003; Rahman *et al.*, 2008; Kaushik *et al.*, 2009).

The percentage of cell adhering to xylene and toluene, apolar solvent, demonstrated hydrophobic cell surface properties. Of all test strains, a significant difference ($P < 0.05$) of cell surface hydrophobicity was observed (Table 8). Seven strains (TISTR 2072, TISTR 2073, TISTR 2074, TISTR 2075, TISTR 2079, TISTR 2081 and TISTR 2082) exhibited high cell surface hydrophobicity ranging from 47.14 to 99.79% and 29.69 to 80.15% in toluene and xylene, respectively, which was higher than that of *E. coli* O157:H7 DMST 12743 (23.27 and 27.54%) and *S.* Typhimurium ATCC 13311 (45.62 and 11.79%). This suggested that the ability of these strains to adhere to epithelial cell was greater than that of pathogens. However, other strains showed lower cell surface hydrophobicity (3.91–10.56% in toluene and 0.42–5.60% in xylene). Cell surface hydrophobicity has been reported for some *Lactobacilli*, *L. crispatus* LT116 and *L. johnsonii* LT171 isolated from crop and ileum of chickens tested in toluene showed high degree of cell hydrophobicity of 92.14 and 85.21%, respectively (Taheri *et al.*, 2009a; Taheri *et al.*, 2009b). While, *L. plantarum* L4 displayed 6.52% in xylene (Kos *et al.*, 2000). These differences in cell surface hydrophobicity could be due to variation in the level of expression of cell surface protein among strains of a species as well as due to environmental conditions which could affect the expression of surface protein (Kaushik *et al.*, 2009).

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Table 7 Autoaggregation and coaggregation ability of *L. plantarum.*

Strains	Aggregation (% ± S.D.)						
	Autoaggregation (h)					Coaggregation (% ± S.D.)	
	1 h	2 h	3 h	4 h	5 h	with *E. coli*	with *S.* Typhimurium
TISTR 2070	7.25 ± 2.37	39.67 ± 3.26	22.55 ± 5.80	56.52 ± 2.65	73.82 ± 0.62 ^{de}	N.D.	N.D.
TISTR 2071	0.56 ± 0.24	50.46 ± 3.16	30.94 ± 0.77	56.79 ± 0.50	63.80 ± 1.35 ^{g}	N.D.	N.D.
TISTR 2072	1.33 ± 1.00	48.00 ± 1.63	54.17 ± 2.89	75.67 ± 1.28	86.97 ± 1.96 ^{a}	18.75 ± 8.44 ^{a}	3.85 ± 3.14 ^{b}
TISTR 2073	1.61 ± 1.08	47.18 ± 2.75	54.97 ± 0.78	68.82 ± 2.48	73.27 ± 8.71 ^{de}	3.68 ± 1.47 ^{b}	3.91 ± 1.56 ^{b}
TISTR 2074	1.06 ± 1.06	51.98 ± 1.52	54.37 ± 0.76	70.11 ± 2.78	76.61 ± 1.04 ^{bcd}	N.D.	N.D.
TISTR 2075	1.58 ± 1.05	60.13 ± 1.51	60.53 ± 2.40	70.00 ± 0.61	75.62 ± 0.86 ^{cd}	9.26 ± 2.14 ^{b}	10.00 ± 5.16 ^{ab}
TISTR 2076	1.43 ± 0.95	51.79 ± 3.17	55.36 ± 0.69	65.48 ± 1.96	68.02 ± 1.05 ^{f}	N.D.	N.D.
TISTR 2077	2.60 ± 1.66	50.65 ± 1.50	60.23 ± 3.46	69.91 ± 1.30	72.77 ± 3.34 ^{de}	N.D.	N.D.
TISTR 2078	14.34 ± 0.74	58.92 ± 1.06	67.60 ± 0.88	70.96 ± 0.42	77.03 ± 0.94 ^{bcd}	8.11 ± 2.21 ^{b}	N.D.
TISTR 2079	6.85 ± 1.54	54.64 ± 1.56	67.49 ± 2.08	70.16 ± 2.79	70.87 ± 1.63 ^{ef}	8.97 ± 3.31 ^{b}	12.16 ± 6.43 ^{a}
TISTR 2080	17.83 ± 2.88	60.93 ± 1.00	70.94 ± 0.84	72.55 ± 1.44	80.68 ± 0.34 ^{b}	N.D.	N.D.
TISTR 2081	10.04 ± 0.77	56.56 ± 0.67	65.49 ± 0.48	72.39 ± 2.47	74.73 ± 1.56 ^{de}	5.88 ± 4.80 ^{b}	5.47 ± 2.99 ^{ab}
TISTR 2082	10.12 ± 4.98	68.77 ± 1.12	72.76 ± 1.12	68.87 ± 2.70	79.66 ± 1.36 ^{bc}	9.03 ± 3.50 ^{b}	10.29 ± 7.78 ^{ab}

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Table 7 (Continued)

Strains	Aggregation (% ± S.D.)						
	Autoaggregation (h)					Coaggregation (% ± S.D.)	
	1 h	2 h	3 h	4 h	5 h	with *E. coli*	with *S.* Typhimurium
E. coli O157:H7 DMST 12743	2.89 ± 2.48	33.37 ± 4.34	67.98 ± 0.55	70.66 ± 1.07	73.481 ± 3.46 ^{de}	-	-
S. Typhimurium ATCC 13311	3.60 ± 1.04	35.14 ± 1.56	74.10 ± 0.92	75.68 ± 2.08	77.22±0.38 ^{bcd}	-	-

N.D.: not detected

-: not determined

Values in the same column with different lowercase letters (a–g) are significantly different by Duncan's multiple range test ($P < 0.05$).

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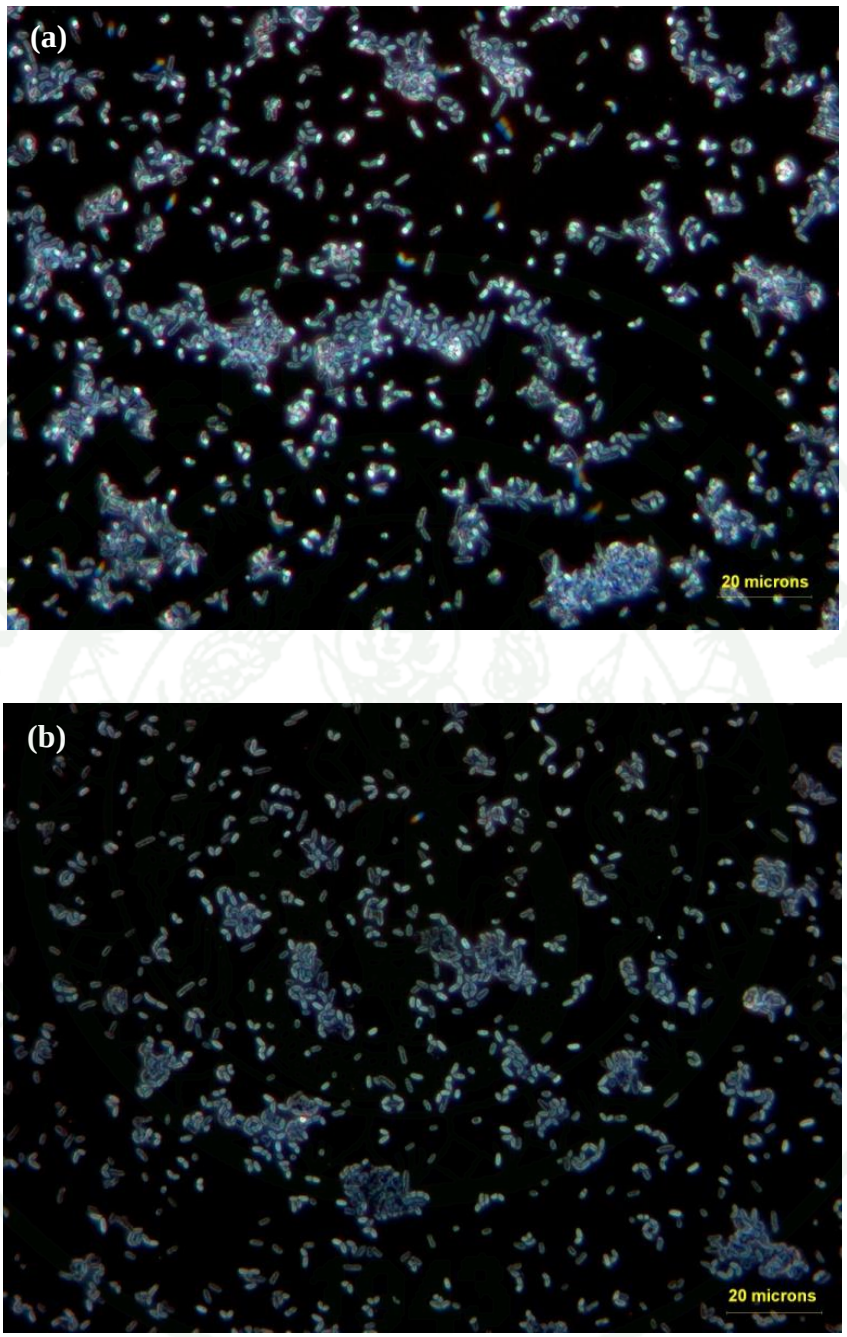

Figure 5 Coaggregation ability between *L. plantarum* TISTR 2082 and (a) *E. coli* O157:H7 DMST 12743; (b) *S.* Typhimurium ATCC 13311.

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Table 8 Cell surface hydrophobicity of *L. plantarum.*

Strains	Cell surface hydrophobicity (% ± S.D.)	
	Toluene	Xylene
TISTR 2070	9.03 ± 3.30 ^{g}	2.47 ± 1.78 ^{g}
TISTR 2071	5.93 ± 5.74 ^{g}	1.53 ± 0.84 ^{g}
TISTR 2072	86.46 ± 2.90 ^{b}	52.63 ± 10.29 ^{b}
TISTR 2073	67.26 ± 4.11 ^{c}	48.76 ± 3.25 ^{bc}
TISTR 2074	57.64 ± 3.25 ^{d}	37.38 ± 7.61 ^{d}
TISTR 2075	99.79 ± 0.08 ^{a}	80.15 ± 3.77 ^{a}
TISTR 2076	10.24 ±5.80 ^{g}	0.65 ± 0.60 ^{g}
TISTR 2077	10.56 ± 5.28 ^{g}	1.71 ± 1.19 ^{g}
TISTR 2078	9.76 ± 8.23 ^{g}	5.60 ± 1.54 ^{fg}
TISTR 2079	55.92 ± 4.97 ^{d}	43.37 ± 1.23 ^{cd}
TISTR 2080	3.91 ± 2.52 ^{g}	0.42 ± 0.36 ^{g}
TISTR 2081	47.14 ± 1.87 ^{e}	43.03 ± 2.68 ^{cd}
TISTR 2082	48.39 ± 2.04 ^{e}	29.69 ± 11.66 ^{e}
E. coli O157:H7 DMST 12743	23.27 ± 0.69 ^{f}	27.54 ± 6.80 ^{e}
S. Typhimurium ATCC 13311	45.62 ± 7.55 ^{e}	11.79 ± 2.53 ^{f}

Values in the same column with different lowercase letters (a–g) are significantly different by Duncan's multiple range test ($P < 0.05$).

2.2 Antibiotic resistance

Antibiotics are utilized by the medical and pharmacological industries to fight pathogenic bacteria. Resistance of probiotic strains to some antibiotics could be used for both preventive and therapeutic purposes in controlling intestinal infections (El-Naggar, 2004). In order to be used as probiotics, lactic acid bacteria must show an ability to resist various antibiotics and subsequently exhibit profitable effects on the health of the host (Sung-Mee and Dong-Soon, 2009).

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Antibiotic disc diffusion susceptibility of all test strains is summarized in Table 9. All strains were totally resistant to kanamycin, ciprofloxacin, streptomycin, oxacillin, vancomycin, polymyxin B and nalidixic acid. Also, they exhibited intermediate resistance to clindamycin, cepfoxitin and bacitracin with inhibition zone ranging from 8 to 14 mm. However, all strains were susceptible to chloramphenicol, rifampin, penicillin, erythromycin, ampicillin, trimethroprim and tetracycline with inhibition zone ranging from 16–35 mm. These results also agree with those of Zhou *et al*. (2005), *L. plantarum* HN045 was resistant to vancomycin, kanamycin, streptomycin, nalidixic acid and polymyxin B and susceptible to penicillin, erythromycin, chloramphenicol, rifampin and tetracycline. Also, Danielsen and Wind (2003) reported that *L. plantarum* was sensitive to penicillin and more resistant to oxacillin and cepfoxitin. *L. plantarum* isolated from Armada cheese was resistant to cepfoxitin, clindamycin, ciprofloxacin and oxacillin (Herreros *et al.*, 2005). Moreover, *L. plantarum* LA22 isolated from fermented fish product was also resistant to bacitracin. Unfortunately, this strain was susceptible to vancomycin (Liasi *et al*., 2009). The susceptibility and resistance to antibiotics of various strains were variable depending on the species (Lim, 2009).

Among the antibiotics, vancomycin resistance is of major concern because it is broadly efficacious against clinical infections caused by multidrug-resistant pathogens (Mathur and Singh, 2005; Zhou *et al.*, 2005). Vancomycin belongs to glycopeptide antibiotics which inhibit the peptidoglycan synthesis. Peptidoglycan is an important structural component of the bacterial cell wall. Some lactic acid bacteria including strains of *L. plantarum*, *L. casei*, *L. rhamnosus* and *L. salivarius* carry intrinsic resistance towards vancomycin. Vancomycin acts by forming a complex with the carboxyterminal D-alanine (D-ala) residue in the building blocks of the bacterial cell wall peptidoglycan. The complex formation prevents the transglycosylation reaction essential to the growth of nascent peptidoglycan polymer (Salminen *et al.,* 1998). Generally, the resistance against vancomycin is based on the modification of the peptidoglycan precursor with the replacement of the terminal D-alanine by D-lactate or in some cases by D-serine (Elisha and courvalin, 1995; Salminen *et al.,* 1998). Many intrinsically vancomycin resistant strains of *Lactobacilli*

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have a long history of safe use as probiotics and there is no indication that vancomycin resistant *Lactobacilli* could transfer the resistance to other bacteria (Saarela *et al.*, 2000). According to the present results, it was observed that all test strains showed an ability of resistance to vancomycin which was in agreement with Charteris *et al*. (1998), Herreros *et al*. (2005) and Zhou *et al*. (2005). In addition, all test strains were sensitive to penicillin (*β*-lactam group) which are bactericidal, became the most widely used therapeutic class of antimicrobial agent because of their broad antibacterial spectrum and excellent safety profile. They inhibit bacterial cell wall synthesis and have a lethal effect on gram-positive bacteria (Herreros *et al.*, 2005). Erythromycin belongs to macorlides group and has similar range of action and efficacy similar to that of penicillin. This group of antibiotics binds to ribosome, blocks protein synthesis (bacteriostatic) and is effective against gram-positive microorganisms (Danielsen and Wind, 2003; Liasi *et al.*, 2009).

2.3 Antimicrobial activity of *L. plantarum*

As shown in Table 10, all strains were found to exhibit antimicrobial activity against *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311 with the inhibition zone in the range of 11.5 to 12.5 mm and 14.0 to 15.5 mm, respectively. Furthermore, CFS (pH 3.65–3.78) obtained from all test strains displayed growth inhibition of *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311. Antimicrobial activity of CFS (pH 5.0) was observed against *E. coli* O157:H7 DMST 12743 (100–200 AU/mL) and *S.* Typhimurium ATCC 13311 (100 AU/mL). Only CFS (pH 6.0) of TISTR 2070, TISTR 2071, TISTR 2072, TISTR 2073 and TISTR 2075 were found to exhibit antimicrobial activity against *E. coli* O157:H7 DMST 12743 (100 AU/mL) but no inhibitory effect against *S.* Typhimurium ATCC 13311 was detected. Based on these results, all adjusted CFS (pH 5.0 and 6.0) were found to lose their antimicrobial activity against the indicator strains. It is suggested that the antimicrobial activity of the test strains relies on acidity, lactic acid or other organic acids being produced (Tsai *et al.*, 2004; Lin *et al.*, 2006; Lapsiri *et al.*, 2011).

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Table 9 Antibiotic susceptibility of the test strains of *L. plantarum.*

Strains	Inhibition zone diameter (mm ± S.D.)							
	P (10 units)	FOX (30 µg)	VA (30 µg)	B (10 IU/IE/UI)	AM (10 µg)	OX (1 µg)	K (30 µg)	S (10 µg)
TISTR 2070	21 ± 1 (S)	14 ± 1 (R)	6 ± 0 (R)	8 ± 0 (R)	35 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2071	20 ± 1 (I)	13 ± 2 (R)	6 ± 0 (R)	8 ± 0 (R)	34 ± 2 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2072	18 ± 0 (I)	8 ± 0 (R)	6 ± 0 (R)	12 ± 1 (R)	35 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2073	21 ± 1 (S)	9 ± 1 (R)	6 ± 0 (R)	14 ± 0 (R)	35 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2074	20 ± 0 (I)	10 ± 1 (R)	6 ± 0 (R)	11 ± 1 (R)	35 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2075	16 ± 0 (I)	9 ± 1 (R)	6 ± 0 (R)	6 ± 0 (R)	35 ± 0 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2076	20 ± 1 (I)	12 ± 1 (R)	6 ± 0 (R)	6 ± 0 (R)	35 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2077	21 ± 0 (S)	13 ± 1 (R)	6 ± 0 (R)	6 ± 0 (R)	34 ± 2 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2078	20 ± 0 (I)	13 ± 2 (R)	6 ± 0 (R)	8 ± 0 (R)	35 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2079	21 ± 2 (S)	11 ± 1 (R)	6 ± 0 (R)	6 ± 0 (R)	33 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2080	20 ± 1 (I)	13 ± 1 (R)	6 ± 0 (R)	12 ± 0 (R)	34 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2081	18 ± 0 (I)	12 ± 2 (R)	6 ± 0 (R)	8 ± 0 (R)	35 ± 1 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)
TISTR 2082	21 ± 1 (S)	11 ± 1 (R)	6 ± 0 (R)	8 ± 0 (R)	33 ± 0 (S)	6 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)

P: Penicillin; FOX: Cepfoxitin; VA: Vancomycin; B: Bacitracin; AM: Ampicillin; OX: Oxacillin; K: Kanamycin; S: Streptomycin

R: resistant; I: intermediate sensitive; S: sensitive

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Table 9 (Continued)

Strains	Inhibition zone diameter (mm ± S.D.)								
	TE (30 µg)	C (30 µg)	E (15 µg)	CC (2 µg)	Cip (5 µg)	NA (30 µg)	RA (5 µg)	TMP (5 µg)	PB (300 IU/IE/UI)
TISTR 2070	20 ± 1 (I)	23 ± 1 (S)	23 ± 1 (S)	14 ± 2 (R)	6 ± 0 (R)	6 ± 0 (R)	19 ± 1 (I)	25 ± 1 (S)	6 ± 0 (R)
TISTR 2071	18 ± 1 (I)	25 ± 0 (S)	23 ± 1 (S)	11 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	21 ± 1 (S)	25 ± 1 (S)	6 ± 0 (R)
TISTR 2072	18 ± 0 (I)	24 ± 0 (S)	23 ± 0 (S)	13 ± 1 (R)	6 ± 0 (R)	6 ± 0 (R)	22 ± 1 (S)	24 ± 0 (S)	6 ± 0 (R)
TISTR 2073	19 ± 0 (I)	28 ± 1 (S)	24 ± 1 (S)	13 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	23 ± 0 (S)	22 ± 0 (S)	6 ± 0 (R)
TISTR 2074	19 ± 1 (I)	25 ± 1 (S)	22 ± 1 (S)	14 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	23 ± 1 (S)	24 ± 1 (S)	6 ± 0 (R)
TISTR 2075	21 ± 1 (S)	22 ± 2 (S)	23 ± 0 (S)	14 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	17 ± 1 (I)	25 ± 0 (S)	6 ± 0 (R)
TISTR 2076	22 ± 1 (S)	22 ± 1 (S)	25 ± 0 (S)	11 ± 1 (R)	6 ± 0 (R)	6 ± 0 (R)	18 ± 1 (I)	25 ± 1 (S)	6 ± 0 (R)
TISTR 2077	22 ± 1 (S)	22 ± 1 (S)	24 ± 1 (S)	11 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	19 ± 1 (I)	24 ± 1 (S)	6 ± 0 (R)
TISTR 2078	21 ± 0 (S)	27 ± 2 (S)	23 ± 1 (S)	13 ± 1 (R)	6 ± 0 (R)	6 ± 0 (R)	21 ± 1 (S)	24 ± 1 (S)	6 ± 0 (R)
TISTR 2079	21 ± 1 (S)	23 ± 2 (S)	25 ± 1 (S)	12 ± 2 (R)	6 ± 0 (R)	6 ± 0 (R)	20 ± 1 (I)	27 ± 0 (S)	6 ± 0 (R)
TISTR 2080	21 ± 0 (S)	24 ± 2 (S)	25 ± 2 (S)	14 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	19 ± 1 (I)	25 ± 1 (S)	6 ± 0 (R)
TISTR 2081	21 ± 1 (S)	24 ± 1 (S)	24 ± 0 (S)	14 ± 1 (R)	6 ± 0 (R)	6 ± 0 (R)	20 ± 1 (I)	25 ± 0 (S)	6 ± 0 (R)
TISTR 2082	20 ± 1 (I)	24 ± 1 (S)	24 ± 1 (S)	12 ± 0 (R)	6 ± 0 (R)	6 ± 0 (R)	18 ± 0 (I)	27 ± 0 (S)	6 ± 0 (R)

TE: Tetracycline; C: Chloramphenicol; E: Erythromycin; CC: Clindamycin; Cip: Ciprofloxacin; NA: Nalidixic acid; RA: Rifampin; TMP: Trimethroprim; PB: Polymyxin

R: resistant; I: intermediate sensitive; S: sensitive

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Table 10 Antimicrobial activity of *L. plantarum* against *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311.

Strains	Antibacterial activities against indicator strains							
	E. coli O157:H7 DMST 12743				*S.* Typhimurium ATCC 13311			
	Agar well diffusion assay (mm ± S.D.)	Spot-on-lawn assay (AU/mL)			Agar well diffusion assay (mm ± S.D.)	Spot-on-lawn assay (AU/mL)		
		CFS*	CFS (pH 5.0)	CFS (pH 6.0)		CFS*	CFS (pH 5.0)	CFS (pH 6.0)
TISTR 2070	12.0 ± 0.2 ^{b}	300	200	100	14.5 ± 0.5 ^{b}	100	0	0
TISTR 2071	12.5 ± 0.1 ^{a}	300	200	100	14.3 ± 0.3 ^{bc}	100	0	0
TISTR 2072	12.0 ± 0.1 ^{b}	300	200	100	14.3 ± 0.3 ^{bc}	200	100	0
TISTR 2073	12.0 ± 0.1 ^{b}	300	200	100	14.0 ± 0.2 ^{bc}	200	100	0
TISTR 2074	12.5 ± 0.1 ^{a}	200	100	0	14.1 ± 0.2 ^{c}	200	100	0
TISTR 2075	12.0 ± 0.1 ^{b}	200	100	100	14.2 ± 0.3 ^{bc}	200	100	0
TISTR 2076	12.0 ± 0.1 ^{b}	100	0	0	15.5 ± 0.5 ^{a}	200	100	0
TISTR 2077	12.0 ± 0.1 ^{b}	100	0	0	14.5 ± 0.5 ^{bc}	100	0	0
TISTR 2078	11.5 ± 0.1 ^{c}	100	0	0	14.5 ± 0.4 ^{bc}	100	0	0
TISTR 2079	12.0 ± 0.1 ^{b}	200	0	0	15.3 ± 0.5 ^{a}	100	0	0
TISTR 2080	12.5 ± 0.1 ^{b}	200	100	0	14.5 ± 0.4 ^{bc}	200	100	0
TISTR 2081	12.0 ± 0.1 ^{b}	100	0	0	14.5 ± 0.4 ^{bc}	200	100	0
TISTR 2082	12.0 ± 0.1 ^{b}	100	0	0	14.5 ± 0.5 ^{bc}	100	0	0

Values with different lowercase letters (a–c) are significantly by Duncan's multiple range test ($P < 0.05$).

* Non-adjusted cell-free supernatant

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2.4 Heat tolerance of *L. plantarum*

A major challenge associated with the application of probiotic cultures in functional foods is the retention of viability during processing. In this respect, the drying of live probiotic strains is a critical step in the preparation of concentrated probiotic food ingredients. The heat tolerance of *L. plantarum* incubated at 65 °C for up to 60 min is shown in Figure 6. *L. plantarum* TISTR 2075 exhibited the highest heat tolerance after heat exposure for 30 min with a survival rate of 98.51% followed by TISTR 2077 (92.55%), TISTR 2070 (89.06%), TISTR 2076 (81.21%) and TISTR 2078 (79.75%). In contrast, *L. plantarum* TISTR 2072, TISTR 2079 and TISTR 2082 were found to be very sensitive to heat with the totally loss of viability. However, no strains remained viable after 60 min of incubation. Compared to Ding and Shah (2007), a higher loss of viability was observed in the encapsulated and free cells of *L. plantarum* after heat treatment at 65 °C for 30 min with approximately 2 and 4 log CFU/mL, respectively. Kim *et al.* (2001) suggested that a temperature at 60 °C was considered as the lethal temperature because the viability of *L. acidophilus* was significantly reduced but not all cells were killed. Additionally, Champagne *et al.* (2005) suggested that temperatures over 65 °C are highly detrimental to probiotic cultures. Therefore, it could be claimed that *L. plantarum* TISTR 2075, TISTR 2077, TISTR 2070, TISTR 2076 and TISTR 2078 are thermotolerant strains (≥ 80% survival).

Heat tolerance of lactic acid bacteria is a complex process involving proteins with different roles in cell physiology, including chaperone activity, ribosome stability, stringent response mediation, temperature sensing and control of ribosomal functions (De Angelis and Gobbetti, 2004; Serrazanetti *et al.*, 2009). The major encountered by cells at high temperature is the denaturation of proteins and their subsequent aggregation. The destabilization of macromolecules (ribosomes and RNA) and alterations of membrane fluidity as responses to heat stress. Interestingly, bacteria that enter into stationary phase develop a general stress resistance and are more resistant to various types of stresses (including subsequent down-stream

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processing and storage) than bacteria in log phase (Kosin and Rakshit, 2010). Guzzo *et al*. (1997) reported that the stationary phase cells survive better after drying because the synthesis of stress protein was induced in this phase and the stress proteins could act as an important role in the cell survival after drying.

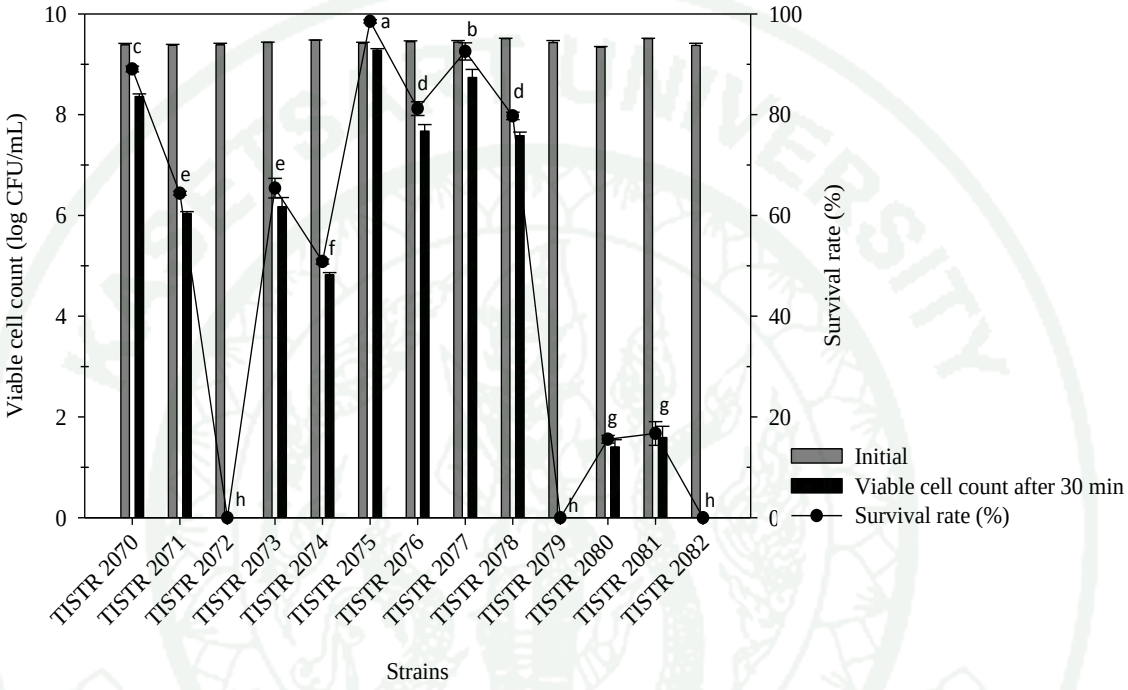

Figure 6 Survival of *L. plantarum* after exposure to 65 °C for 60 min. Values with different lowercase letters (a–h) are significant differently by Duncan's multiple range test ($P < 0.05$).

2.5 Viability of *L. plantarum* after exposure to simulated gastric and small intestinal juices

All of 13 test strains were found to exhibit the tolerance ability to simulated gastric juice at pH 2.0 for 180 min (Table 11). Seven strains (TISTR 2073, TISTR 2074, TISTR 2075, TISTR 2076, TISTR 2077, TISTR 2078 and TISTR 2081) survived under conditions of pH < 2.0 with a survival rate of 47.80–71.20%, while the other strains (TISTR 2070, TISTR 2071, TISTR 2072, TISTR 2079, TISTR 2080 and

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TISTR 2082) were sensitive to acid conditions and their viability was found to be completely destroyed after an exposure of 180 min. TISTR 2073 exhibited the highest tolerance with a viability loss of approximately 2.8 log CFU/mL. This was supported by Pennacchia *et al*. (2004) that *Lactobacillus* spp. exhibited a survival rate of 60–80% in PBS pH 2.5 for 3 h at 37 °C. However, according to Michida *et al*. (2006), *L. plantarum* NCIMB 8826 was found to have a higher loss of viability of approximately 8 log cycles after exposure to simulated gastric juice at pH 2.0 for 30 min.

The acid-tolerant strains of *L. plantarum* were selected to test their ability to survive in simulated small intestinal juice with 0.45% bile salt which was considered as sufficient concentration to determine any resistant strains (Buntin *et al.*, 2008). As shown in Table 12, all strains selected were quite stable in simulated small intestinal juice with 0.45% bile salt for 240 min with viable cell reduction less than 27.00%. Three strains (TISTR 2073, TISTR 2077 and TISTR 2081) were observed to be the most bile tolerant with survival rates of 84.90, 89.96 and 89.31%, respectively. A similar finding was previously reported by Kacem *et al*. (2006) where *L. plantarum* OL9 and OL36 isolated from fermented olives showed the highest tolerance (65 and 59%, respectively). The viability of *L. plantarum* NCIMB 8826 isolated from human saliva was similarly found to decrease by 1.9 log CFU/mL (Patel *et al.*, 2004a). According to Serrazanetti *et al.* (2009), the small intestinal juice tolerance of probiotic bacteria was strain dependent. Bile resistance of *Lactobacillus* spp. is related to the specific enzyme activity of bile salt hydrolase (BSH) which helps the hydrolysis of conjugated bile and thus reduces its toxic effects (du Toit *et al.*, 1998).

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Table 11 Viability of *L. plantarum* during exposure to simulated gastric juice.

Strains	Viable cell count (log CFU/mL ± S.D.)					Survival rate (% ± S.D.)
	Initial	After exposure				
		30 min	60 min	90 min	180 min	180 min
TISTR 2070	9.87 ± 0.52	6.90 ± 0.27	6.28 ± 0.07	4.95 ± 0.99	0.00 ± 0.00	0.00 ± 0.00 ^{e}
TISTR 2071	9.98 ± 0.11	7.49 ± 0.54	5.66 ± 0.84	0.00 ± 0.00	0.00 ± 0.00	0.00± 0.00 ^{e}
TISTR 2072	9.98 ± 0.65	8.26 ± 0.79	7.30 ± 0.56	6.58 ± 0.83	0.00 ± 0.00	0.00 ± 0.00 ^{e}
TISTR 2073	9.73 ± 0.28	9.01 ± 0.69	8.68 ± 0.68	8.23 ± 0.70	6.93 ± 0.82	71.20 ± 8.43 ^{a}
TISTR 2074	10.10 ± 0.55	9.07 ± 0.35	7.20 ± 0.36	6.92 ± 0.53	6.44 ± 0.82	63.78 ± 8.13 ^{abc}
TISTR 2075	9.75 ± 0.36	9.08 ± 0.94	8.18 ± 0.93	7.32 ± 0.67	4.66 ± 0.12	47.80 ± 1.20 ^{d}
TISTR 2076	9.97 ± 0.52	9.23 ± 0.26	7.75 ± 0.75	7.65 ± 0.58	5.39 ± 0.84	54.05 ± 8.40 ^{cd}
TISTR 2077	9.84 ± 0.38	8.73 ± 0.54	8.00 ± 0.24	7.26 ± 0.53	6.39 ± 0.60	64.98 ± 6.10 ^{ab}
TISTR 2078	10.03 ± 0.40	8.44 ± 0.83	6.42 ± 0.13	5.84 ± 0.07	5.47 ± 0.18	54.56 ± 1.82 ^{bcd}
TISTR 2079	9.59 ± 0.52	4.42 ± 0.51	3.59 ± 0.63	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 ^{e}
TISTR 2080	9.85 ± 0.54	4.37 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 ^{e}
TISTR 2081	9.87 ± 0.28	9.39 ± 0.06	8.24 ± 0.97	7.40 ± 0.92	5.46 ± 0.33	55.32 ± 3.30 ^{bcd}
TISTR 2082	9.87 ± 0.17	5.57 ± 0.59	5.18 ± 0.26	4.91 ± 0.10	0.00 ± 0.00	0.00 ± 0.00 ^{e}

Values with different lowercase letters (a–e) are significantly different by Duncan's multiple range test ($P < 0.05$).

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Table 12 Viability of acid-tolerant strains of *L. plantarum* during exposure to simulated small intestinal juice.

Strain	Viable cells (log CFU/mL ± S.D.)		Survival rate (% ± S.D.)
	Initial	After exposure	
TISTR 2073	9.66 ± 0.09	8.19 ± 0.02	84.90 ± 0.02 [b]
TISTR 2074	9.78 ± 0.04	7.14 ± 0.05	73.14 ± 0.36 [d]
TISTR 2075	9.95 ± 0.05	7.72 ± 0.08	77.51 ± 0.90 [c]
TISTR 2076	9.37 ± 0.02	7.30 ± 0.08	78.00 ± 0.10 [c]
TISTR 2077	9.63 ± 0.17	8.72 ± 0.02	89.96 ± 0.98 [a]
TISTR 2078	9.56 ± 0.21	7.29 ± 0.15	76.79 ± 0.73 [c]
TISTR 2081	9.72 ± 0.09	8.65 ± 0.10	89.31 ± 0.55 [a]

Values with different lowercase letters (a–d) are significantly different by Duncan's multiple range test ($P < 0.05$).

From the above results, it could be indicated that *L. plantarum* TISTR 2075 isolated from fermented vegetables was found to meet all the criteria outlined above and could be considered as potential probiotic. This strain showed strong autoaggregation and cell surface hydrophobicity related to the adhesion ability to intestinal cells and it also had positive coaggregation with *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311 linked to the ability to interact closely with pathogens. In addition, the strain was resistant to some antibiotics tested which belonged to the major classes of antibiotics used in human clinical therapy. Furthermore, it had antimicrobial activity against both pathogens and could survive under gastrointestinal tract conditions. Additionally, it was able to withstand a high temperature of 65 °C for 30 min which is a desirable characteristic for industrial strains as it could have a better chance of remaining viable during the drying process required for prolonged storage. Furthermore, this strain was reconfirmed by partial 16S rDNA sequence analysis as *L. plantarum* which was recognized as GRAS

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(Appendix F). Therefore, *L. plantarum* TISTR 2075 may be regarded as a potential probiotic candidate and used as the starter culture for further study.

3. Effect of cereal flour on the viability of *L. plantarum* under gastrointestinal tract conditions

3.1 Effects of soybean, sesame and Job's Tears flour on the viability of *L. plantarum* under simulated gastric juice (pH 2.0)

In the present study, 13 strains of *L. plantarum* were evaluated for their tolerances to simulated gastric juice pH 2.0 (Figure 7). It was observed that all test strains exhibited certain resistance abilities to gastric juice for 180 min. Among all strains tested, 7 strains (*L. plantarum* TISTR 2073, TISTR 2074, TISTR 2075, TISTR 2076, TISTR 2077, TISTR 2078 and TISTR 2081) appeared to be more capable to survive and their viable cell populations were higher than 4.5 log CFU/mL. *L. plantarum* TISTR 2073 was found to be the most tolerant with a reduction rate of 28.80 % (from 9.73 to 6.93 log CFU/mL). However, there was no significant difference ($P > 0.05$) among the survival of *L. plantarum* TISTR 2073 (71.2%), TISTR 2074 (63.8%) and TISTR 2077 (65.0%). Whereas, the lower survival rate of approximately 47.8–55.3% was observed in *L. plantarum* TISTR 2081, TISTR 2078, TISTR 2076 and TISTR 2075. Comparing to Michida *et al*. (2006) and Valerio *et al*. (2006), a higher loss of viability of approximately 5 log CFU/mL was observed in *L. plantarum* NCIMB 8826 and *L. plantarum* ITM21B after exposure to gastric juice pH 2.0 for 180 min. Unfortunately, the other six strains (*L. plantarum* TISTR 2070, TISTR 2071, TISTR 2072, TISTR 2079, TISTR 2080 and TISTR 2082) were susceptible to gastric condition and the number of viable cell decreased to undetectable levels after exposure for 180 min.

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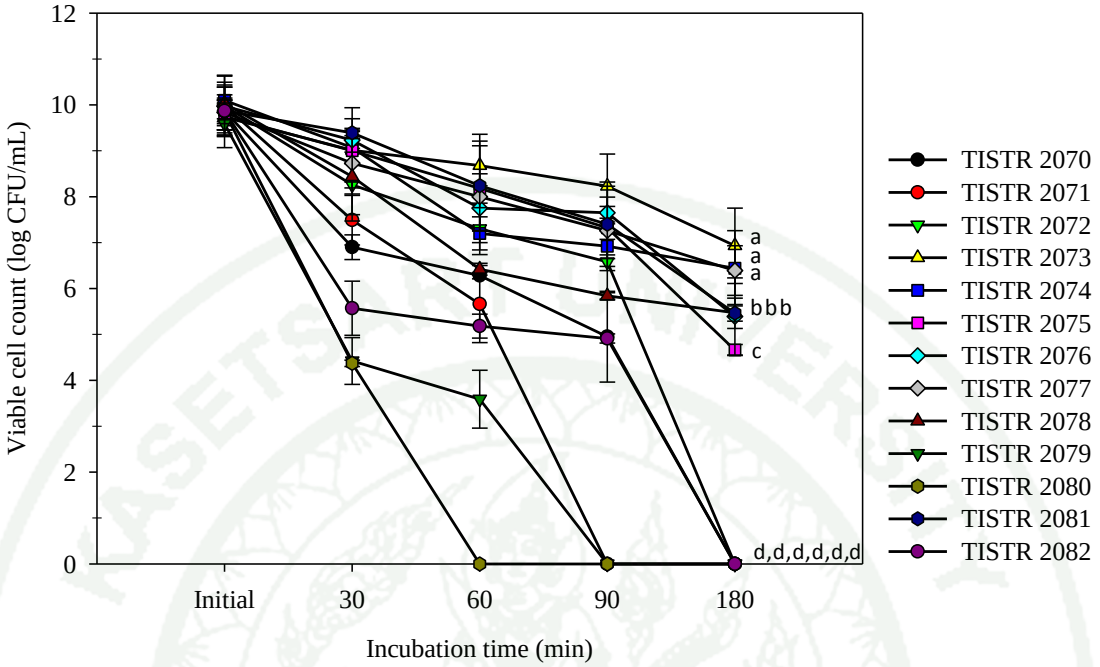

Figure 7 Viability of *L. plantarum* during exposure to simulated gastric juice. Values with different lowercase letters (a–d) are significant different by Duncan's multiple range test ($P < 0.05$).

The effect of cereal flour on the survival of all test strains during exposure to the acid juice is shown in Table 13. The addition of soybean, sesame and Job's Tears exhibited significantly ($P < 0.05$) improved acid tolerance of all test strains, particularly six acid-sensitive strains and their tolerance level depended on the cereal types. Soybean flour was found to be the best protective compound followed by sesame flour and Job's Tears flour. Compared to control group, the viability of all test strains was considerable improved by approximately 2.13–9.45, 2.08–9.27 and 2.10–9.17 log CFU/mL in the presence of soybean, sesame and Job's Tears flour, respectively.

Among the test strains, *L. plantarum* TISTR 2073, TISTR 2074, TISTR 2075, TISTR 2076, TISTR 2077, TISTR2078 and TISTR 2081 appeared to be most capable to survive gastric transit in the present of cereal flour with high viability of

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approximately 87.87 to 97.67% (Figure 8). The acid tolerance of all test strains suspended in the cereal flour could be explained by the protective action of component such as protein, fat, polysaccharide, prebiotic carbohydrates, metabolizable sugars and free amino acids (Charalampopoulos *et al.*, 2002; Charalampopoulos *et al.*, 2003; Corcoran *et al.*, 2005; Michida *et al.*, 2006; Valerio *et al.*, 2006; Wang *et al.*, 2007). According to the previous study of Valerio *et al*. (2006), a slight decrease in cell population of *L. plantarum* ITM21B (from 7.86 to 7.22 log CFU/mL) was observed when artichoke was added as food carriers resulting from the presence of prebiotic carbohydrates in artichokes as well as the protective action of the fat content from olives and milk. This result was in close agreement with the finding of Wang *et al*. (2009) that the buffering capacity of peanut protein significantly improved the viability of *L. plantarum* P9 in simulated gastric juice. In addition, metabolizable sugar and free amino nitrogen in malt and barley extract also resulted in higher survival rate of *L. plantarum* NCIMB 8826 (Charalampopoulos *et al.*, 2003; Michida *et al.*, 2006).

A number of mechanisms have been shown to confer acid stress, i.e., the ATPase-triggered proton pumping, the ADI system and the GAD system. The main mechanism for controlling intracellular pH is the F_0F_1-ATPase. In the presence of metabolizable sugar, it provides ATP to F_0F_1-ATPase via glycolysis, enabling proton exclusion and thereby enhancing survival during gastric transit (McDonald *et al.*, 1990; Sanders *et al.*, 1999; van de Guchte *et al.*, 2002; Cotter and Hill, 2003; Corcoran *et al.*, 2005). From the results, sugar content and buffering capacity of the cereals may have a synergistic effect on the gastrointestinal tolerance. As shown in Table 14, soybean showed the highest values of all composition tested, while there was no significant different ($P > 0.05$) in total sugars and reducing sugars in sesame and Job's Tears and the buffering capacity of sesame was higher than Job's Tears. Charalampopoulos *et al.* (2003) suggested that the higher concentrations of total sugar than reducing sugar could be attributed to the presence of sucrose or other soluble oligosaccharides. Additionally, glucose and maltose from cereals (malt, barley and wheat) exert a protective effect of *L. plantarum* NCIMB 8826 viability under acidic condition, which could partly justify the protective effect of cereals. Also, the higher

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buffering capacity of soybean compared to that of sesame and Job's Tears would justify the enhanced protective effect of soybean.

The effect of sugars such as glucose and fructose on the survival of *Lactobacilli* in acidic environments has been widely investigated by Corcoran *et al*. (2004) who found that the survival of *L. paracasei* and *L. gasseri* and the well-known *L. rhamnosus* GG exposed to simulated gastric juice was highly enhanced in the presence of glucose and fructose and that viability varied among species. The protective effect of metabolize sugars has also been confirmed by Chalalampopoulos *et al*. (2003) who demonstrated that the viability of *L. plantarum* NCIMB 8826, *L. acidophilus* NCIMB 8821 and *L. reuteri* NCIMB 11951 increased in the presence of malt, wheat and barley extracts and depended on the strain. The authors associated this result with the chemical composition of cereals mainly with the presence of soluble sugars and to a lesser extent with their free amino nitrogen content. In particular, some plant-based nutrients are important in promoting the growth of probiotics and it has been suggested that fruit juice and vegetables are healthy foods which could serve as a good medium for cultivating probiotics because they are rich in antioxidants, vitamins, metabolizable sugars, fibers and minerals.

From SEM results, it was clear that a high cell density of *L. plantarum* TISTR 2073 was entrapped on the fiber structure of soybean, sesame and Job's Tears (Figure 9). The roughness of the structure may offer protection to the cells in the acid condition (Valerio *et al.*, 2006). It was obvious that chemical composition and physical structure of cereal flour appeared to play a major role in the protective effects (Ranadheera *et al.*, 2010). Therefore, the cereal flour could be used as means for probiotics to enhance their stability during exposure to gastric digestion (Lapsiri and Wanchaitanawong, 2012a).

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Table 13 Viability of *L. plantarum* during exposure to simulated gastric juice in the presence of soybean, sesame and Job's Tears flour.

Strains	Cereal flour	Viable cell count (log CFU/mL ± S.D.)				
		Initial	30 min	60 min	90 min	180 min
TISTR 2070	Control	9.87 ± 0.52	6.90 ± 0.27	6.28 ± 0.07	4.95 ± 0.99	0.00 ± 0.00 [d]
	Soybean		9.81 ± 0.13	9.64 ± 0.03	9.56 ± 0.13	9.45 ± 0.02 [a]
	Sesame		9.23 ± 0.07	9.12 ± 0.06	8.91 ± 0.21	8.27 ± 0.01 [b]
	Job's Tears		9.29 ± 0.15	7.07 ± 0.11	6.03 ± 0.13	5.69 ± 0.11 [c]
TISTR 2071	Control	9.98 ± 0.11	7.49 ± 0.54	5.66 ± 0.84	0.00 ± 0.00	0.00 ± 0.00 [d]
	Soybean		9.78 ± 0.08	9.62 ± 0.12	9.63 ± 0.04	9.41 ± 0.01 [a]
	Sesame		9.64 ± 0.06	9.70 ± 0.02	9.59 ± 0.05	8.12 ± 0.10 [b]
	Job's Tears		9.21 ± 0.11	8.85 ± 0.04	8.05 ± 0.08	7.14 ± 0.03 [c]
TISTR 2072	Control	9.98 ± 0.65	8.26 ± 0.79	7.30 ± 0.56	6.58 ± 0.83	0.00 ± 0.00 [b]
	Soybean		9.82 ± 0.15	9.65 ± 0.03	9.44 ± 0.11	9.17 ± 0.01 [a]
	Sesame		9.79 ± 0.13	9.51 ± 0.11	9.24 ± 0.09	9.16 ± 0.02 [a]
	Job's Tears		9.71 ± 0.01	9.41 ± 0.06	9.45 ± 0.11	9.17 ± 0.01 [a]
TISTR 2073	Control	9.73 ± 0.28	9.01 ± 0.69	8.68 ± 0.68	8.23 ± 0.70	6.93 ± 0.82 [b]
	Soybean		9.67 ± 0.02	9.69 ± 0.12	9.19 ± 0.04	9.06 ± 0.15 [a]
	Sesame		9.38 ± 0.11	9.43 ± 0.03	9.13 ± 0.05	9.01 ± 0.18 [a]
	Job's Tears		9.52 ± 0.02	9.36 ± 0.11	9.37 ± 0.01	9.03 ± 0.07 [a]

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Table 13 (Continued)

Strains	Cereal flour	Viable cell count (log CFU/mL ± S.D.)				
		Initial	30 min	60 min	90 min	180 min
TISTR 2074	Control	10.10 ± 0.55	9.07 ± 0.35	7.20 ± 0.36	6.92 ± 0.53	6.44 ± 0.82 [b]
	Soybean		10.08 ± 0.22	9.91 ± 0.21	9.66 ± 0.02	9.48 ± 0.04 [a]
	Sesame		9.56 ± 0.05	9.31 ± 0.01	8.88 ± 0.22	8.57 ± 0.11 [a]
	Job's Tears		9.60 ± 0.05	8.74 ± 0.06	8.77 ± 0.07	8.55 ± 0.15 [a]
TISTR 2075	Control	9.75 ± 0.36	9.08 ± 0.94	8.18 ± 0.93	7.32 ± 0.67	4.66 ± 0.12 [d]
	Soybean		9.80 ± 0.12	9.86 ± 0.02	9.55 ± 0.05	9.06 ± 0.03 [a]
	Sesame		9.33 ± 0.09	9.40 ± 0.06	8.58 ± 0.02	7.96 ± 0.09 [b]
	Job's Tears		9.98 ± 0.04	9.35 ± 0.06	8.83 ± 0.13	8.23 ± 0.15 [c]
TISTR 2076	Control	9.97 ± 0.52	9.23 ± 0.26	7.75 ± 0.75	7.65 ± 0.58	5.39 ± 0.84 [b]
	Soybean		9.99 ± 0.02	9.82 ± 0.06	9.66 ± 0.09	9.49 ± 0.04 [a]
	Sesame		9.25 ± 0.04	8.69 ± 0.10	8.68 ± 0.37	8.76 ± 0.08 [a]
	Job's Tears		9.55 ± 0.11	9.52 ± 0.04	9.36 ± 0.09	9.27 ± 0.01 [a]
TISTR 2077	Control	9.84 ± 0.38	8.73 ± 0.54	8.00 ± 0.24	7.26 ± 0.53	6.39 ± 0.60 [b]
	Soybean		9.87 ± 0.02	9.66 ± 0.16	9.33 ± 0.07	9.03 ± 0.02 [a]
	Sesame		9.89 ± 0.02	9.66 ± 0.06	9.47 ± 0.07	9.09 ± 0.01 [a]
	Job's Tears		9.66 ± 0.02	9.63 ± 0.03	9.32 ± 0.04	9.16 ± 0.03 [a]

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Table 13 (Continued)

Strains	Cereal flour	Viable cell count (log CFU/mL ± S.D.)				
		Initial	30 min	60 min	90 min	180 min
TISTR 2078	Control	10.03 ± 0.40	8.44 ± 0.83	6.42 ± 0.13	5.84 ± 0.07	5.47 ± 0.18 [c]
	Soybean		9.82 ± 0.06	9.77 ± 0.10	9.50 ± 0.02	9.37 ± 0.01 [ab]
	Sesame		9.69 ± 0.06	9.56 ± 0.07	9.34 ± 0.04	9.06 ± 0.37 [b]
	Job's Tears		9.73 ±0.07	9.78 ± 0.02	9.69 ± 0.12	9.61 ± 0.06 [a]
TISTR 2079	Control	9.59 ± 0.52	4.42 ± 0.51	3.59 ± 0.63	0.00 ± 0.00	0.00 ± 0.00 [c]
	Soybean		9.47 ± 0.02	9.38 ± 0.05	9.08 ± 0.02	8.09 ± 0.02 [a]
	Sesame		9.78 ± 0.08	8.66 ± 0.07	8.73 ± 0.05	8.04 ± 0.09 [a]
	Job's Tears		8.57 ± 0.03	7.09 ± 0.03	6.75 ± 0.03	4.93 ± 0.09 [b]
TISTR 2080	Control	9.85 ± 0.54	4.37 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 [c]
	Soybean		9.70 ± 0.07	9.64 ± 0.10	9.44 ± 0.06	9.23 ± 0.33 [a]
	Sesame		9.51 ± 0.04	9.41 ± 0.05	9.49 ± 0.00	9.27 ± 0.02 [a]
	Job's Tears		9.68 ± 0.03	8.36 ± 0.02	7.17 ± 0.01	6.30 ± 0.20 [b]
TISTR 2081	Control	9.87 ± 0.28	9.39 ± 0.06	8.24 ± 0.97	7.40 ± 0.92	5.46 ± 0.33 [c]
	Soybean		9.84 ± 0.03	9.78 ± 0.01	9.67 ± 0.01	9.64 ± 0.06 [a]
	Sesame		9.78 ± 0.06	9.63 ± 0.21	9.56 ± 0.07	9.33 ± 0.16 [ab]
	Job's Tears		9.60 ± 0.01	9.42 ± 0.04	9.28 ± 0.04	9.08 ± 0.10 [b]

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Table 13 (Continued)

Strains	Cereal flour	Viable cell count (log CFU/mL ± S.D.)				
		Initial	30 min	60 min	90 min	180 min
TISTR 2082	Control	9.87 ± 0.17	5.57 ± 0.59	5.18 ± 0.26	4.91 ± 0.10	0.00 ± 0.00 [d]
	Soybean		9.71 ± 0.17	9.50 ± 0.01	9.59 ± 0.21	9.40 ± 0.05 [a]
	Sesame		9.11 ± 0.02	8.88 ± 0.11	8.56 ± 0.03	7.96 ± 0.02 [b]
	Job's Tears		9.05 ± 0.07	8.57 ± 0.02	8.04 ± 0.01	7.12 ± 0.01 [c]

Values in the same column of each test strain with different lowercase letter (a–d) are significantly different by Duncan's multiple range test ($P < 0.05$).

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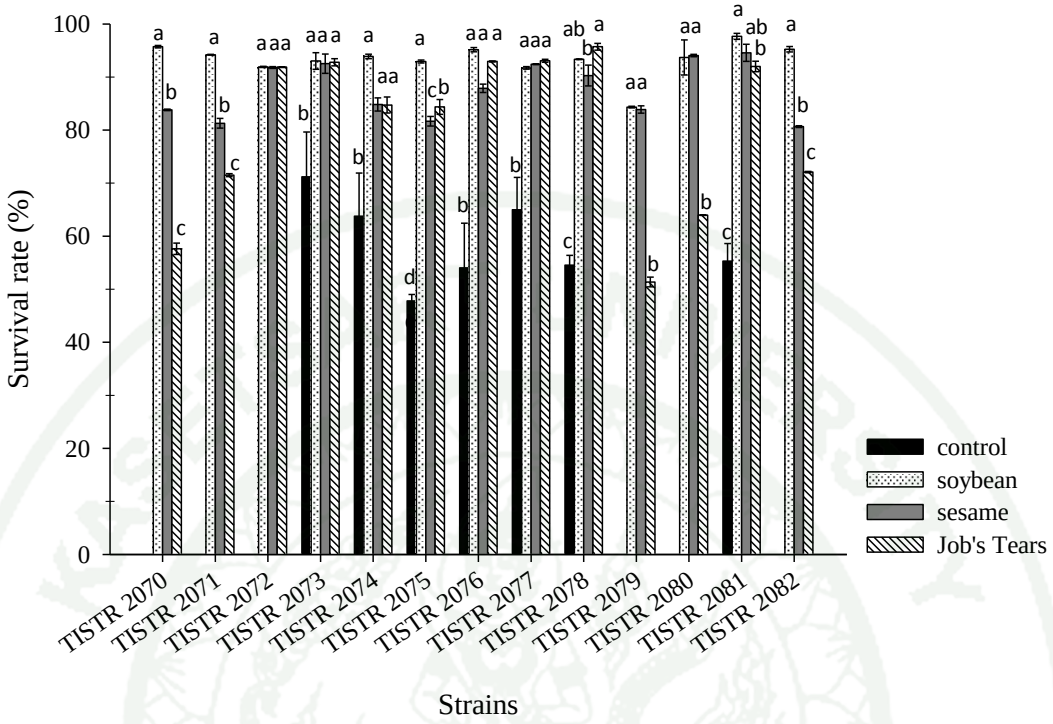

Figure 8 Survival of *L. plantarum* with and without soybean, sesame and Job's Tears flour under exposure to simulated gastric juice. Different lowercase letters (a–d) of each strain are significantly different by Duncan's multiple range test ($P < 0.05$).

Table 14 Chemical composition of sterile cereal suspensions (10% w/v).

Characteristic	Soybean	Sesame	Job's Tears
pH	6.40 ± 0.02 [a]	6.04 ± 0.02 [b]	6.37 ± 0.01 [a]
Total sugars (g/L)	18.41 ± 0.62 [a]	6.74 ± 1.12 [b]	8.83 ± 0.67 [b]
Reducing sugars (g/L)	0.66 ± 0.02 [a]	0.16 ± 0.01 [b]	0.15 ± 0.01 [b]
Buffering capacity (mmol/pH.L)	20.05 ± 0.07 [a]	5.25 ± 0.07 [b]	4.00 ± 0.14 [c]

Values in the same row with different lowercase letters (a–c) are significantly different by Duncan's multiple range test ($P < 0.05$).

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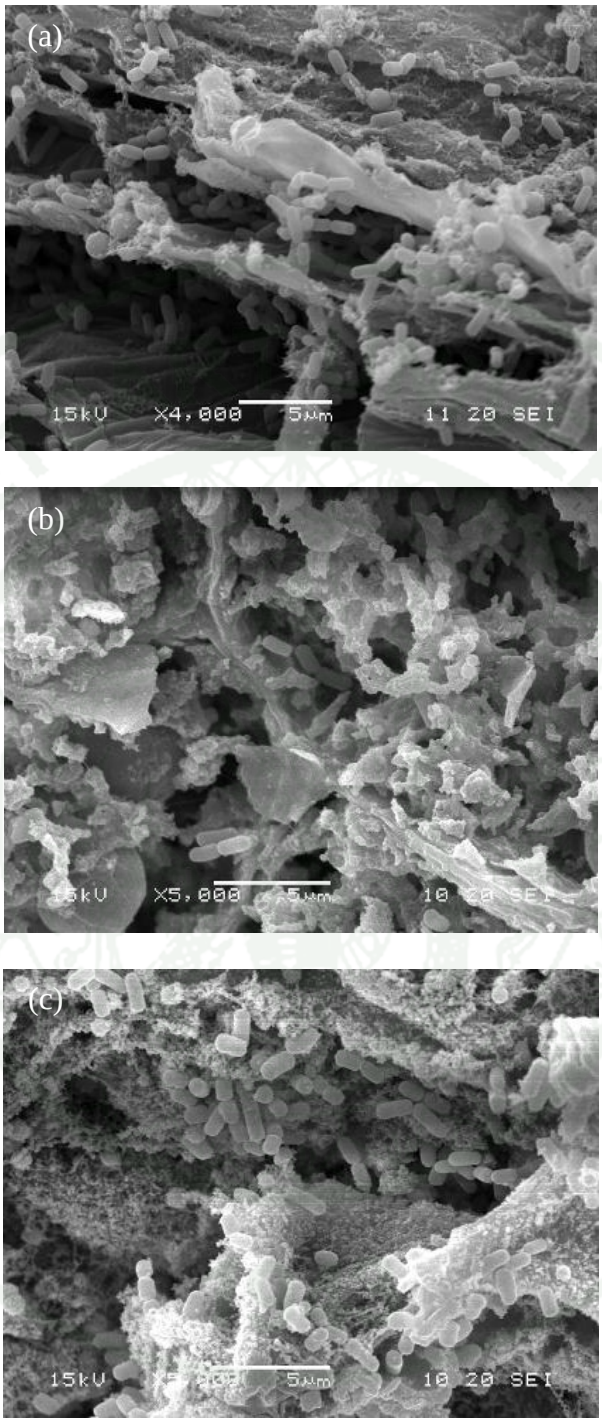

Figure 9 Scanning electron micrographs of *L. plantarum* TISTR 2073 in the presence of (a) soybean (4000×); (b) sesame (5000×) and (c) Job's Tears (5000×).

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3.2 Effects of soybean, sesame and Job's Tears on viability of *L. plantarum* under simulated small intestinal juice

The capability to survive under small intestinal juice in the human intestine in order to take up residence and multiply in human large intestine is another important characteristic of lactic acid bacteria to be used as probiotic dietary adjuncts. In probiotic selection, small intestinal tolerance is of potentially more importance than gastric survival. With the development of new delivery systems and use of specific foods, evidence clearly demonstrates that acid-sensitive strains can be buffered through the stomach. However, to exert a positive effect on the health and well being of a host, probiotics need to colonise and survive in the small intestine (Havenaar *et al.*, 1992).

In the present study, *L. plantarum* was carried out for 240 min exposure to simulated small intestinal juice (pH 8.0) with 0.45% bile salt considered as sufficient as to allow for an assessment of resistant strains (Huang and Adams, 2004; Michida *et al.*, 2006; Buntin *et al.*, 2008). As shown in Figure 10 and Table 15, in the control experiments, all test strains were found to be capable to survive during exposure to simulated small intestinal juice with 0.45% bile salt for 240 min and the reduction of the viable cells count ranged from 0.26 to 2.64 log CFU/mL. Of 13 strains, a slightly decreased viability was observed in *L. plantarum* TISTR 2080 and TISTR 2070 and their cell population slightly decreased from 9.95 to 9.69 log CFU/mL (97.28 % survival) and 9.27 to 8.86 log CFU/mL (95.42 % survival), respectively. Survival of the other eleven strains ranged from 73.14 to 89.86%. Consistent with Huang and Adams (2004), most of test 13 strains of *Propionibacterium freudenreichii* and *P. acidopropionici* demonstrated high level of small intestinal transit tolerance with a small reduction in their viability (0.2 to 1 log CFU/mL) in the presence of bile salt. *L. casei* 212.3, *L. fermentum* KLD and *L. rhamnosus* GG also retained viability during simulated small intestinal transit for up to 4 h considered as intrinsically tolerant. In contrast, *B. adolescentis* 15703T showed a progressive reduction in viability and was considered intrinsically sensitive.

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In the present of soybean, sesame and Job's Tears flour, all cereals exhibited similar effect on viability of all test strains during 240 min exposure in the bile juice. Compared to control group, the cell population of most strains decreased slightly, except for *L. plantarum* TISTR 2081 and TISTR 2074 which were significantly improved ($P < 0.05$) in viability and their survival rates were 94.80 to 99.65% (~10^9 CFU/mL) and 83.11 to 87.39% (~10^8 CFU/mL), respectively. Moreover, it was observed that Job's Tears flour was found to be more effective in protecting cells from harsh environmental conditions than soybean flour and sesame flour and most of test strains could survive better in the presence of Job's Tears flour. These results were in accordance with Michida *et al*. (2006) that the addition of malt and barley showed considerable increased in viability of *L. plantarum* NCIMB 8826 from 7.59 to 7.72 and 7.58 to 7.67 log CFU/mL, respectively.

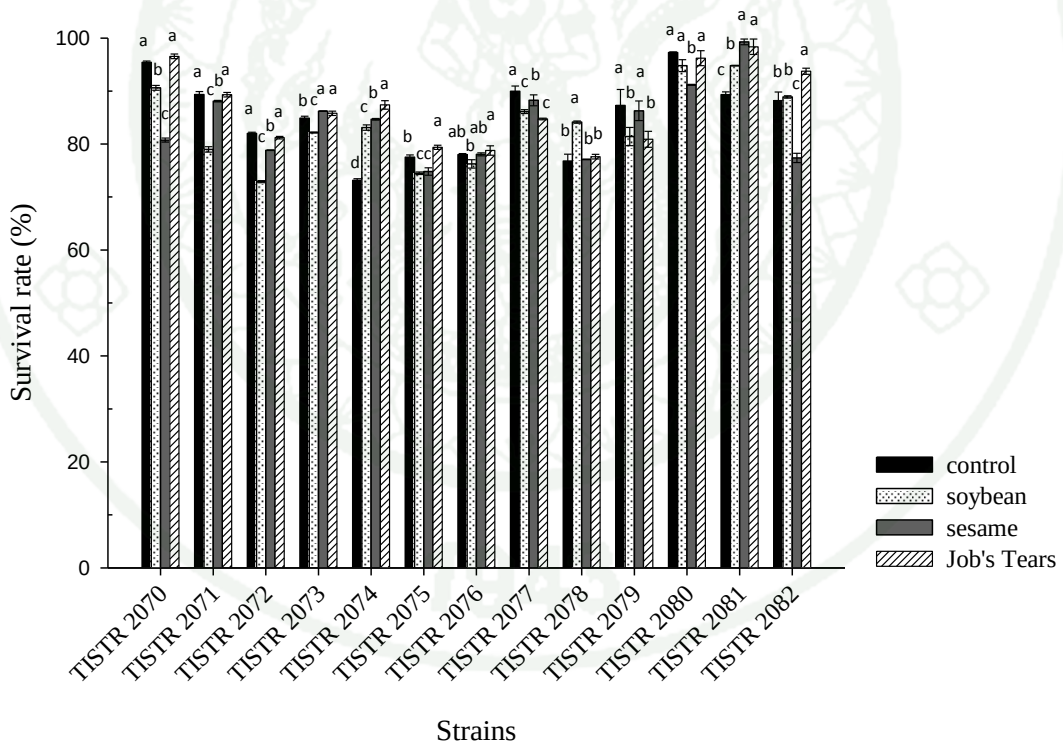

Figure 10 Effect of soybean, sesame and Job's Tears flour on viability of *L. plantarum* during exposure to simulated small intestinal juice. Different lowercase letters (a–c) of each strain are significantly different by Duncan's multiple range test ($P < 0.05$).

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Table 15 Viability of *L. plantarum* during exposure to simulated small intestinal juice in the presence of cereal flour.

Strains	Viable cell count (log CFU/mL ± S.D.)				
	Initial	Control	Soybean	Sesame	Job's Tears
TISTR 2070	9.27±0.03	8.86±0.19 ^{a}	8.40±0.04 ^{b}	7.49±0.04 ^{c}	8.95±0.04 ^{a}
TISTR 2071	9.92±0.04	8.85±0.04 ^{a}	7.84±0.05 ^{c}	8.74±0.01 ^{b}	8.86±0.04 ^{a}
TISTR 2072	9.60±0.01	7.88±0.06 ^{a}	7.00±0.02 ^{c}	7.57±0.01 ^{b}	7.80±0.02 ^{a}
TISTR 2073	9.66±0.04	8.19±0.01 ^{b}	7.94±0.01 ^{c}	8.33±0.00 ^{a}	8.29±0.04 ^{a}
TISTR 2074	9.78±0.04	7.14±0.05 ^{d}	8.13±0.05 ^{c}	8.28±0.02 ^{b}	8.55±0.08 ^{a}
TISTR 2075	9.95±0.03	7.72±0.08 ^{b}	7.41±0.02 ^{c}	7.44±0.07 ^{c}	7.90±0.04 ^{a}
TISTR 2076	9.37±0.02	7.30±0.00 ^{ab}	7.15±0.07 ^{b}	7.31±0.03 ^{ab}	7.38±0.08 ^{a}
TISTR 2077	9.63±0.17	8.72±0.02 ^{a}	8.30±0.04 ^{c}	8.50±0.10 ^{b}	8.16±0.01 ^{c}
TISTR 2078	9.56±0.21	7.29±0.15 ^{b}	8.04±0.02 ^{a}	7.37±0.00 ^{b}	7.42±0.04 ^{b}
TISTR 2079	9.59±0.67	8.57±0.02 ^{a}	7.81±0.16 ^{b}	8.27±0.18 ^{a}	7.76±0.14 ^{b}
TISTR 2080	9.95±0.04	9.69±0.03 ^{a}	9.43±0.11 ^{a}	9.07±0.01 ^{b}	9.57±0.14 ^{a}
TISTR 2081	9.72±0.09	8.65±0.01 ^{c}	9.21±0.01 ^{b}	9.65±0.05 ^{a}	9.56±0.14 ^{a}
TISTR 2082	9.30±0.10	8.17±0.05 ^{b}	8.27±0.02 ^{b}	7.20±0.08 ^{c}	8.72±0.05 ^{a}

Values in the same row with different lowercase letters (a–c) are significantly different by Duncan's multiple range test ($P < 0.05$).

In addition, the acid-tolerant strains (*L. plantarum* TISTR 2073, TISTR 2074, TISTR 2075, TISTR 2076, TISTR 2077, TISTR 2078 and TISTR 2081) were selected to test for their viability after sequential exposure to simulated gastric (180 min) and small intestinal juices (240 min). Similarly, survival of all test strains was improved in the presence of the cereal flour over that of free cell. Interestingly, during exposure to the bile juice, all test strains were quite stable with survival ranging from 92 to 100% in the present of cereal flour and 84 to 100% in the absence of cereal flour (Figure 11–12). Moreover, these results showed that the addition of the cereal flour could greatly enhance tolerance of *L. plantarum* TISTR 2073, TISTR 2075 and TISTR 2081 to gastric and small intestinal transit. Results revealed that all test strains

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have adaptability in unfavorable conditions (Succi *et al.*, 2005). This was probably because stress responses may be used to improve the survival of microorganisms in stressful condition (Cruz *et al.*, 2010). When microorganism is pre-exposed to one stress such as acid condition, the surviving cells can tolerate better a subsequent unfavourable environment (adverse conditions of the gastrointestinal tracts) (Burns *et al.*, 2008; Buriti *et al.*, 2010). Serrazanetti *et al*. (2009) suggested that responses are different and vast depend on the microorganisms nature and on the environmental stress considered. Lactic acid bacteria respond to stress in a very specific way dependent on the species, on the strains and on the type of stress.

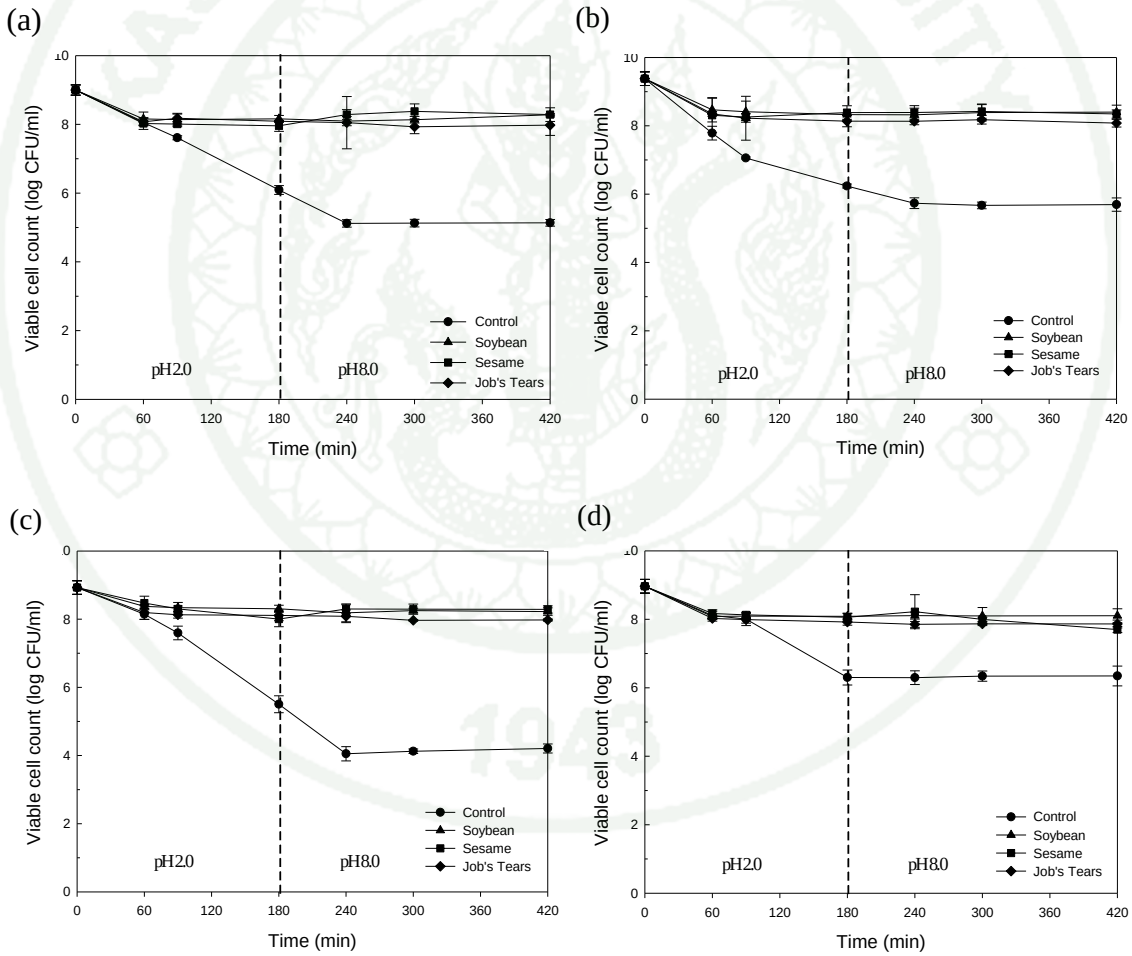

Figure 11 Viability of selected strains during sequential exposure to simulated gastric and small intestinal juice in the absence and presence of soybean, sesame and Job's Tears flour (a) *L. plantarum* TISTR 2073; (b) TISTR 2074; (c) TISTR 2075 and (d) TISTR 2076.

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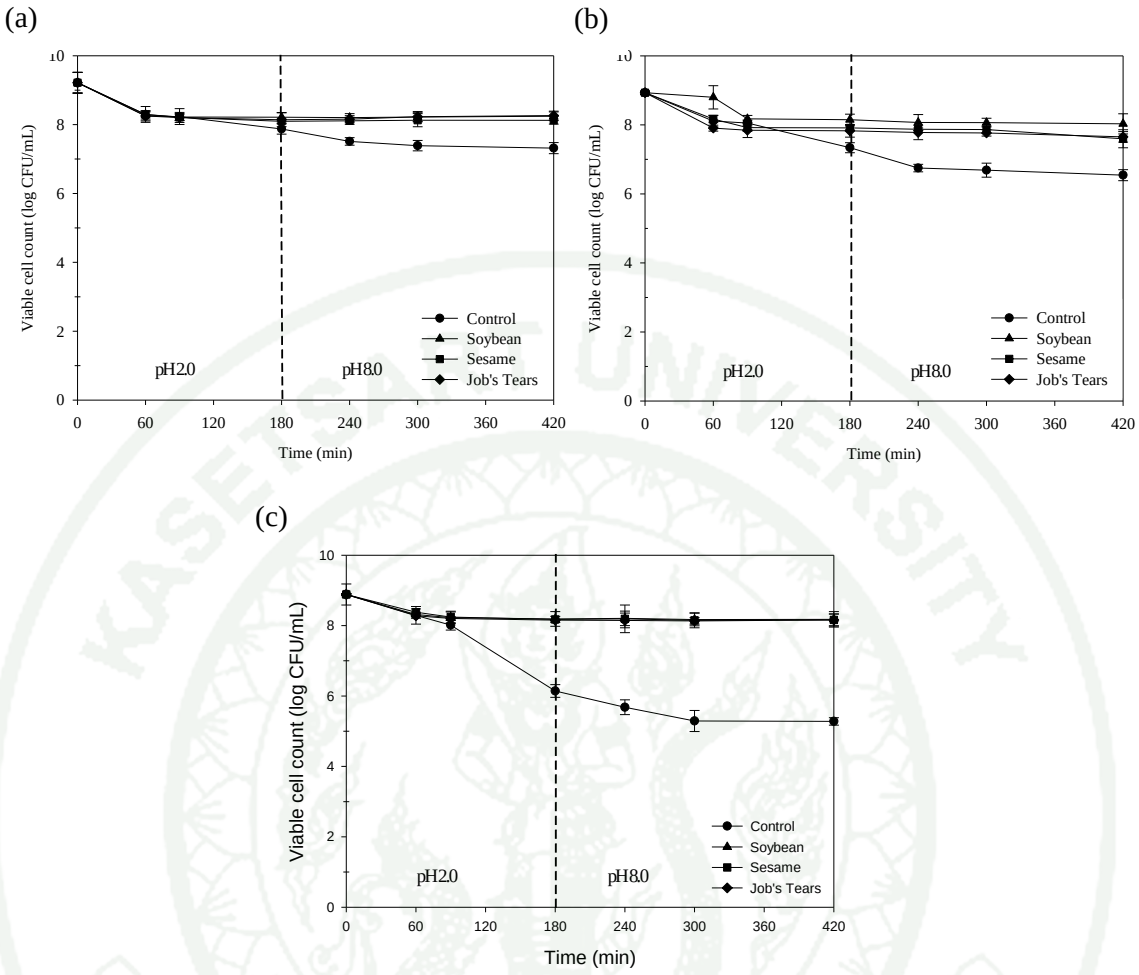

Figure 12 Viability of selected strains during sequential exposure to simulated gastric and small intestinal juice in the absence and presence of soybean, sesame and Job's Tears flour (a) TISTR 2077; (b) TISTR 2078 and (c) TISTR 2081.

4. Fermentation of cereal extracts by *L. plantarum* TISTR 2075

In the present study, soymilk and Job's Tears extracts supplemented with sesame were used as culture media for the growth of *L. plantarum* TISTR 2075. As shown in Figure 13–14, *L. plantarum* grew well in soymilk and Job's Tears extracts providing the viable cell number of 8.28 and 7.73 log CFU/mL, respectively, after 24 h fermentation. In order to obtain higher cell fermentation, various glucose contents

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(1–20% w/v) were added. In soymilk fermentation, high viable cell number of 8.69–8.96 log CFU/mL was achieved. The highest viable cell number was observed in 10% glucose (8.96 log CFU/mL) which was not significant difference ($P > 0.05$) from 5% glucose (8.86 log CFU/mL). In fermented soymilk supplemented with 1 and 2% sesame, the addition of glucose showed no significant difference ($P > 0.05$) in viable cell count. The similarly results were also observed in Job's Tears extract fermentation. The highest viable cell number of 8.37 log CFU/mL was obtained with the addition of 15% glucose, however it was not different from 1% glucose (8.33 log CFU/mL) (Appendix J). Also, higher glucose contents did not stimulate the growth of strain in fermented Job's Tears extract supplemented with 1 and 2% sesame. These results were in agreement with Timbuntam *et al*. (2006) that there was no significant difference in the viable cell count of *Lactobacillus* spp. FCP2 between 3 and 13% (w/v) sugar in sugar-cane juice. This was probably because higher initial glucose concentration gave rise to an increase of the lag phase and a decrease in the specific growth rate due to a decrease in a_w in the system promoted by large amounts of a water-binding substance (Senthuran *et al.*, 1999; Shirai *et al.*, 2001; Timbuntam *et al.*, 2006). The obtained results indicated that soymilk and Job's Tears extracts could be used as culture media for the growth of *L. plantarum* TISTR 2075. The addition of glucose 1% (w/v) exerted beneficial effects to stimulate the growth of lactic acid bacteria.

As shown in Figure 15, after 24 h soymilk fermentation, the decrease in pH values of < 3.4 was observed in the addition of glucose lower than that of control (pH > 4.0), while the increase in soluble calcium was also obtained. Higher degrees of soluble calcium of 73.0–112.0 mg/L were observed in the addition of glucose. The growth of lactic acid bacteria can lower pH values in varying levels through the production of organic acids during fermentation, particularly lactic acid and acetic acid, related to the increase in calcium solubility (Lopez *et al.*, 2000; Tang *et al.*, 2007). Furthermore, greater degree of soluble calcium was 103.0–127.6 and 122.5–145.3 mg/L in 1 and 2% sesame supplementation, respectively. The similarly results were also observed in fermented Job's Tears extract. However, soluble

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calcium in Job's Tears extract was found to be lower than soymilk (Table 16). The highest soluble calcium of 9.5 mg/L was observed in fermented Job's Tears extract supplemented with 2% sesame and 20% glucose (Figure 16). This was in agreement with the report of Tang *et al*. (2007) that a significant increase ($P < 0.05$) in soluble calcium of 89.3 and 87% was observed in fermentation of soymilk with *L. acidophilus* ATCC4962 and *L. casei* ASCC 290, respectively. The increase in calcium solubility was related to lowered pH associated with production of lactic and acetic acid (Lopez *et al.*, 2000; Tang *et al.*, 2007). There are several mechanisms correlated with microbial activities affecting an increase of mineral solubility including (i) pH decrease, (ii) degradation of the insoluble metal-binding macromolecules such as phytate and (iii) production of the soluble metal-chelating compounds such as organic acids (Bergqvist *et al.*, 2005). Lopez *et al*. (2000) reported that fermentation of *Leuconostoc mesenteroides* strain 38 in whole wheat flour medium established the degradation of phytic acid and the production of lactic acid lead to greater calcium solubility.

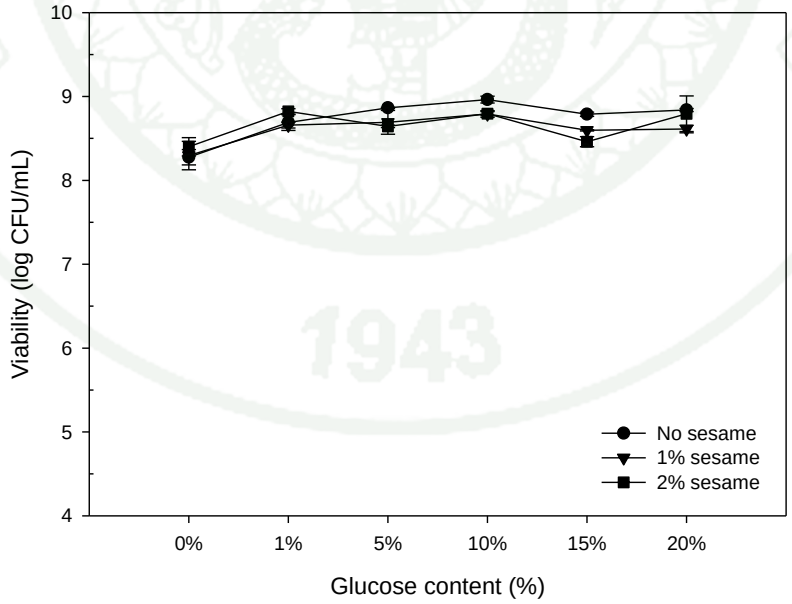

Figure 13 Effect of glucose content on the viability of *L. plantarum* TISTR 2075 in soymilk fermentation.

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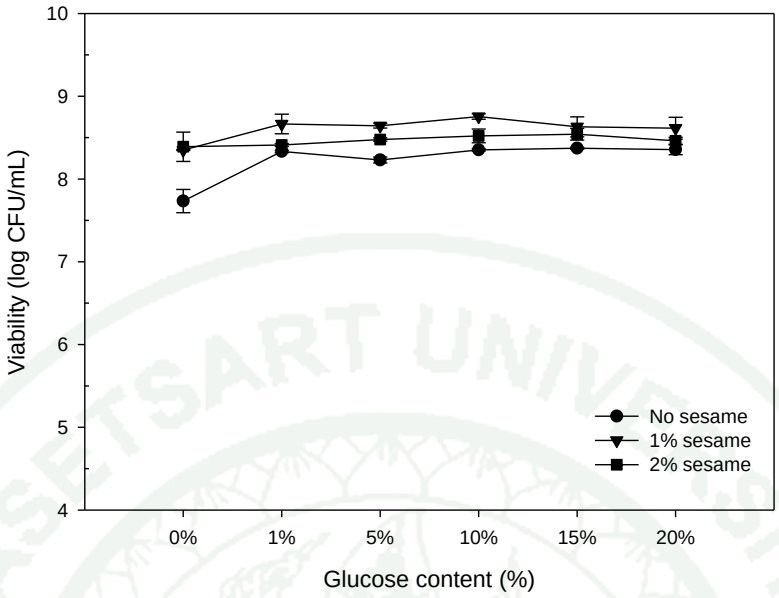

Figure 14 Effect of glucose content on the viability of *L. plantarum* TISTR 2075 in Job's Tears extract fermentation.

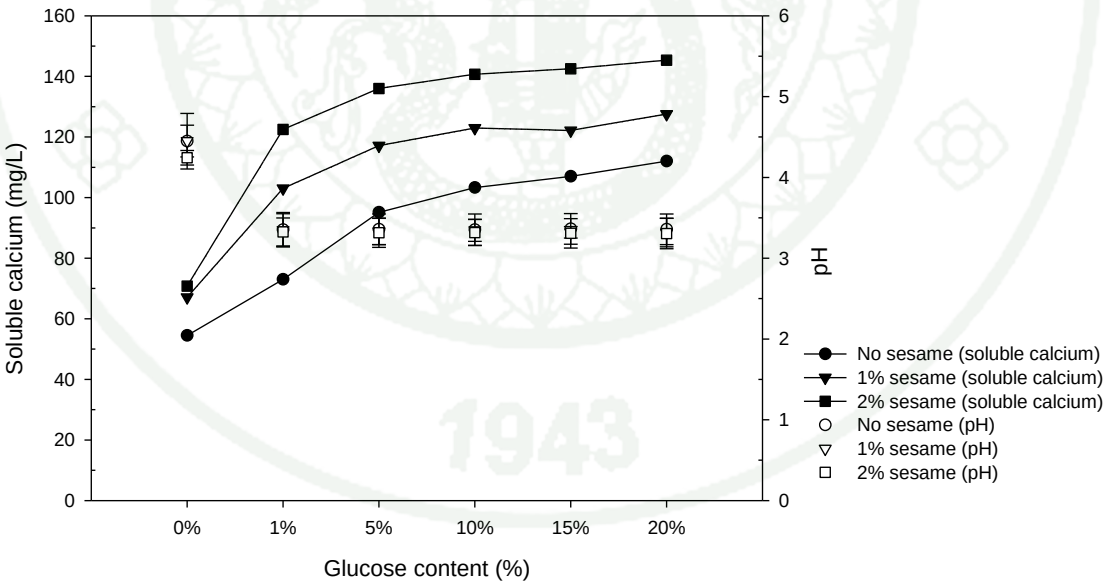

Figure 15 Effect of glucose content on pH and soluble calcium in soymilk fermentation.

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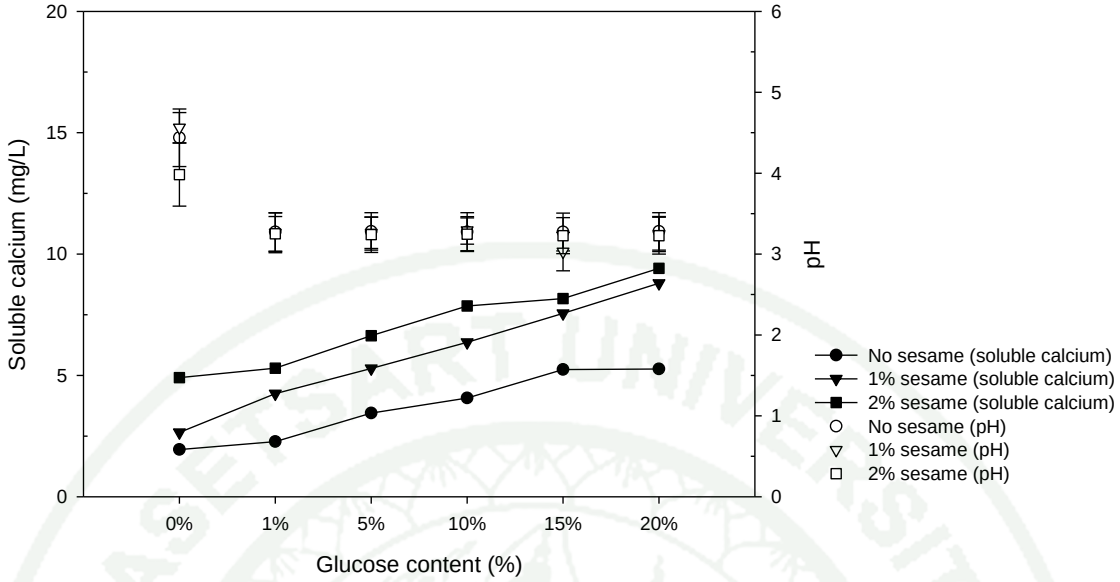

Figure 16 Effect of glucose content on pH and soluble calcium in Job's Tears extract fermentation.

Table 16 Initial calcium content of soymilk and Job's Tears extracts supplemented with 1 and 2% sesame before fermentation.

Conditions	Initial calcium content (mg/L)
Soymilk without sesame supplementation	45.28
Soymilk supplemented with 1% sesame	46.35
Soymilk supplemented with 2% sesame	47.10
Job's Tears extract without sesame supplementation	1.88
Job's Tears extract supplemented with 1% sesame	2.43
Job's Tears extract supplemented with 2% sesame	3.37

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5. Spray drying of *L. plantarum* TISTR 2075

5.1 Survival of *L. plantarum* TISTR 2075 after spray drying using different protective agents

In the preliminary experiment, fermented soymilk and Job's Tears extracts supplemented with 1% sesame was mixed with 20% MD used as carrier prior to spray drying at air inlet and outlet temperatures of 130 and 70 °C, respectively. After spray drying, the survival of *L. plantarum* TISTR 2075 in spray-dried soymilk and Job's Tears extract powders was 74.2 and 77.8%, respectively (Appendix I). For further experiments, fermented Job's Tears juice was therefore used to examine the protective effect of various protectants on the viability of *L. plantarum* TISTR 2075 after spray drying and during storage.

Of 7 protectants; Prot, Tre, Fib and GA significantly ($P < 0.05$) enhanced the viability of *L. plantarum* TISTR 2075 with survival rate of 97.6, 93.3, 84.0 and 82.3%, respectively (Figure 17). In contrast, the addition of Pal, Iso and Asc showed no significant different ($P > 0.05$) in survival rate compared to control (MD). As can be seen from the results, the presence of each protectant enhanced the cell viability with different degrees of protective effect resulting in different survival rates. The protective effect of protectant is probably due to their stabilization effect on cell membrane constituents resulting in the improvement of viability. During drying process, it is known that water removal can lead to de-stabilization of the structural integrity of cellular components causing the loss of cellular viability and activity (Ananta *et al.*, 2005; Yadav *et al.*, 2009; Golowczyc *et al.*, 2011a). The carriers provide a protective effect on the cells since they create a thick wall matrix encapsulating the cells inside microparticle and they could react with and stabilize cellular structures during drying (Fu and Chen, 2011). Cellular structure damage mainly DNA or RNA, protein and enzyme damage due to desiccation is prevented by the addition of protectant as they replace the water binding sites (Crowe *et al.*, 1992; Teixeira *et al.*, 1996; Potts, 2001; Meng *et al.*, 2008; Santivarangkna *et al.*, 2008b; Silva *et al.*, 2011). This results in less damage to the cells during exposure to harsh

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temperature and desiccated condition. After rehydration in saline, the cells were found localized within the microparticle (Figure 18).

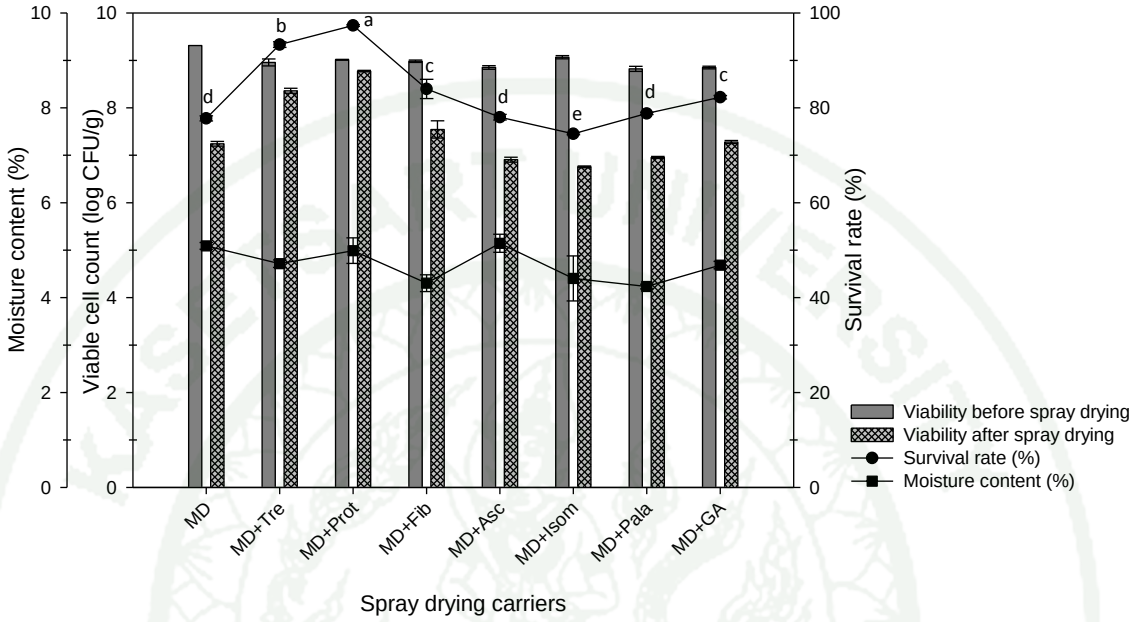

Figure 17 Viable cell count, survival rate and moisture content of *L. plantarum* TISTR 2075 after spray-dried with various protectants. Values with different lowercase letters (a–e) are significant different by Duncan's multiple range test ($P < 0.05$).

In this experiment, Prot was found to be a great protective effect on the viability of *L. plantarum* TISTR 2075. This result was supported by Golowczyc *et al*. (2011) that skim milk powder could maintain the viability of *L. plantarum* 83114 and *L. kefir* 8321 with a lower loss of viability of only 0.11 and 0.29 log CFU/mL, respectively. Consistent with Reddy *et al*. (2009), protein is capable of preventing cellular injury by stabilizing cell membrane *L. plantarum* CFR 2191 with the survival rate of 100% was observed when nonfat skim milk (NFSM) was used as spray-dried carrier. Moreover, the combination of soy protein isolate (SPI)+lactose (Lac) or SMP+MD provided 100% survival of *B. lactis* BB12 after subjected to a two-step drying (Chávez and Ledeboer, 2007). The protective capability of protein may be due to a reaction between the carboxyl groups of the bacterial proteins and the amino

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group of the protectant stabilizing the proteins structure of cells (Zhao and Zhang, 2005). Additionally, protein may form a protective coating on the cell wall proteins (Gharsallaoui *et al.*, 2007; Meng *et al.*, 2008) and creating a structure easy to rehydrate after drying (Silva *et al.*, 2011).

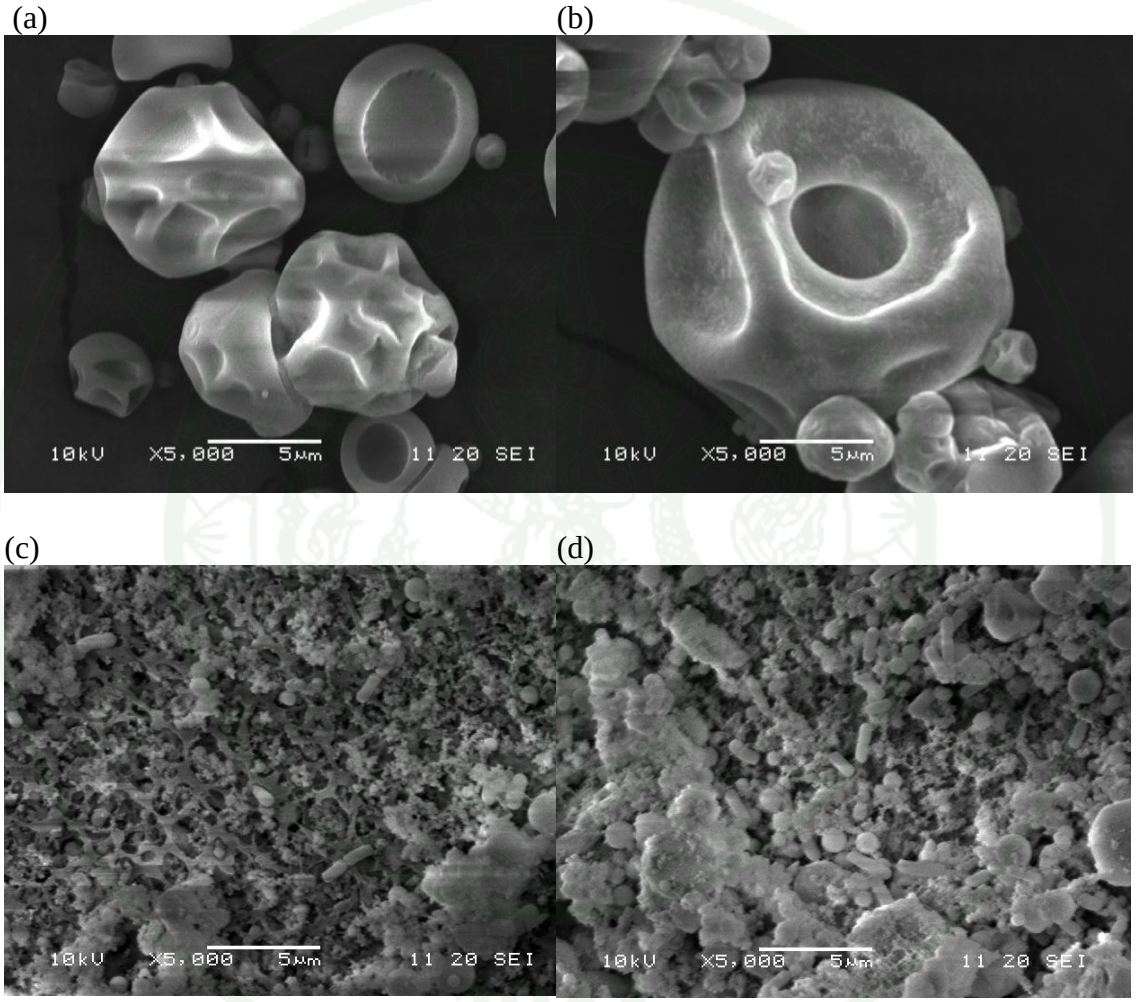

Figure 18 Scanning electron micrographs of spray-dried *L. plantarum* TISTR 2075 with (a) MD-Tre; (b) MD-Prot and (c–d) after rehydration in 0.85% NaCl.

Also, the results revealed that trehalose (Tre) increased survival of *L. plantarum* TISTR 2075 after spray drying. The same tendency was also observed in the report of Sunny-Roberts and Knorr (2009) that a cell viability loss of 0.17 and 0.64 log CFU/mL was detected for *L. rhamnosus* LGG and *L. rhamnosus* E800,

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respectively. It was proved by many researchers that trehalose enhances desiccation tolerance in lactic acid bacteria due to the stabilization of membranes and proteins by replacing the water around polar residues within these macromolecular structures upon thermal stress (Crowe *et al.*, 1996; Crowe *et al.*, 2001; Morgan *et al.*, 2006; Santivarangkna *et al.*, 2008b; Fu and Chen, 2011). The greater flexibility in the glycosidic bond between the two D-glucose molecules, as compared to other disaccharides, may allow trehalose to conform to the irregular polar groups of macromolecules (França *et al*. 2007).

Fib and GA, non-digestible polysaccharide acting as prebiotic (Michel *et al.* 1998; Madley, 2001), were used as protectants in this study. MD+Fib and MD+GA were also found to have a good protection with the viability of 84.0 and 82.3%, greater than control (MD). These results were in concordance with several researchers who used dietary fiber or GA as the protectants in spray drying process. According to the reports of Ananta *et al*. (2005), dietary fiber such as raftilose®P95 and polydextrose (PDX) could be incorporated with RSM exhibited ~40–50% survival after spray drying. Furthermore, a combination of RSM and PDX or inulin in a 1:1 ratio resulted in a high number of *L. rhamnosus* GG up to 10^9 CFU/g (Corcoran *et al.*, 2004). Also, GA was reported to enhance the survival of *L. paracasei* NFBC 338 up to 3 log cycles (Kearney *et al.*, 2009). Consistent with Desmond *et al*. (2002), GA could protect probiotic cultures of *L. paracasei* NFBC338 during spray drying providing 10-fold greater survival rate than skim milk (control).

Finally, ascorbic acid (Asc) did not show significant protective effect on in the strain viability comparing to MD. This result was in agreement with the report of Hamsupo (2005) that no protective effect on the viability of *L. reuteri* KUB-AC5 was observed after spray drying. Asc could act as antioxidant and might be able to protect the dried cell during storage but ascorbic acid can be toxic for the cells. This cytotoxic effect of ascorbic acid may be due to the generation of hydroxyl radicals, which can attack and oxidize biological molecules. Then, the viable cell number can be reduced (Champagne *et al.*, 1991).

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5.2 Effect of spray drying on the probiotic properties

The ability of the spray-dried *L. plantarum* TISTR 2075 to tolerate to simulated gastrointestinal tract conditions and antimicrobial activity against foodborne pathogens, *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311, was investigated. As shown in Figure 19, *L. plantarum* TISTR 2075 retained the ability to tolerate after exposure to simulated gastric juice pH 2.0 for 180 min. The strain in MD+Tre and MD+Prot exhibited the highest tolerance with the survival rate of 61.2 and 59.3%, respectively, followed by MD+GA (51.9% survival rate). The decrease in viability was more pronounced ($P < 0.05$) in MD+Fib and MD with the survival rate of 45.4 and 44.3%, respectively. Comparing with the survival rate of *L. plantarum* TISTR 2075 (47.8% survival) under exposure to acid condition (control), the acid tolerance ability of strain was not affected by spray drying process. The addition of Tre, Prot and GA could stimulate the viability of strain during exposure to acid condition.

Moreover, the spray-dried *L. plantarum* TISTR 2075 in various carriers were also tested for their ability to survive in simulated small intestinal juice pH 8.0 with 0.45% bile salt, As shown in Figure 20, the strain in all spray-dried carriers were stable in 0.45% bile salt for 240 min with survival rate of 82.4–86.2% higher than in control (77.8% survival). This could be indicated that spray drying process did not affect the ability of the strain to tolerate to bile salt condition. Furthermore, there was a significant ($P < 0.05$) increase in viability of the strain when Tre, Prot and GA were used. Similar finding was previously reported by Bucio *et al.* (2005) that no reduction in viability of spray-dried *L. plantarum* 44a was observed after exposure to intestinal simulation pH 8.0 for 2 h.

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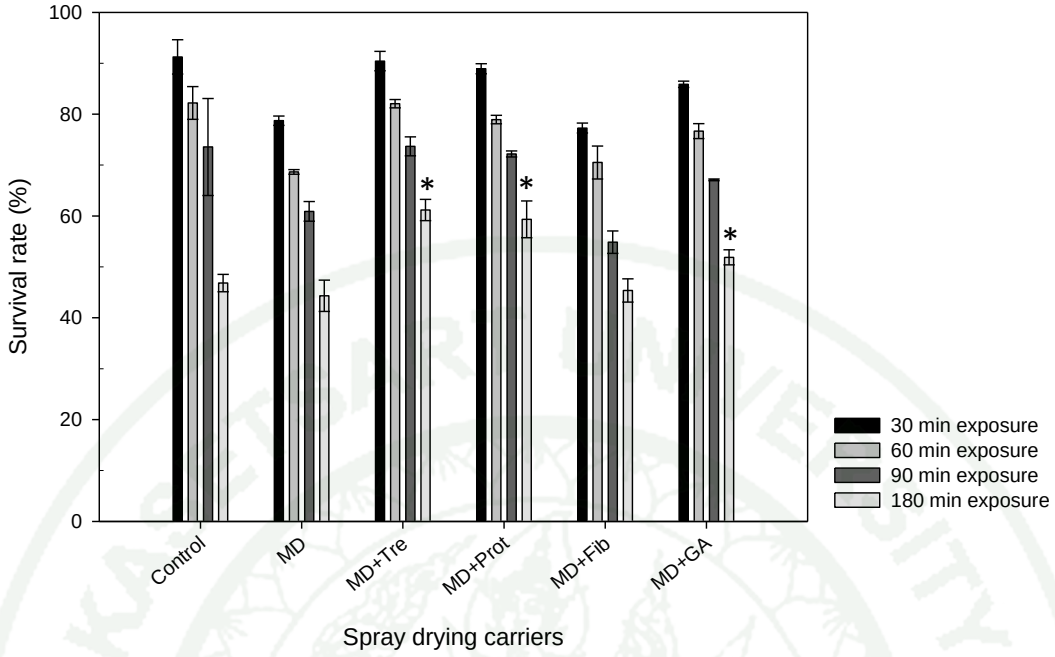

Figure 19 Survival rate of spray-dried *L. plantarum* TISTR 2075 in various carriers after exposure to simulated gastric juice. Survival rates (%) of strains after exposure for 180 min were compared with control, * $P < 0.05$ (Student's *t*-test, two tailed).

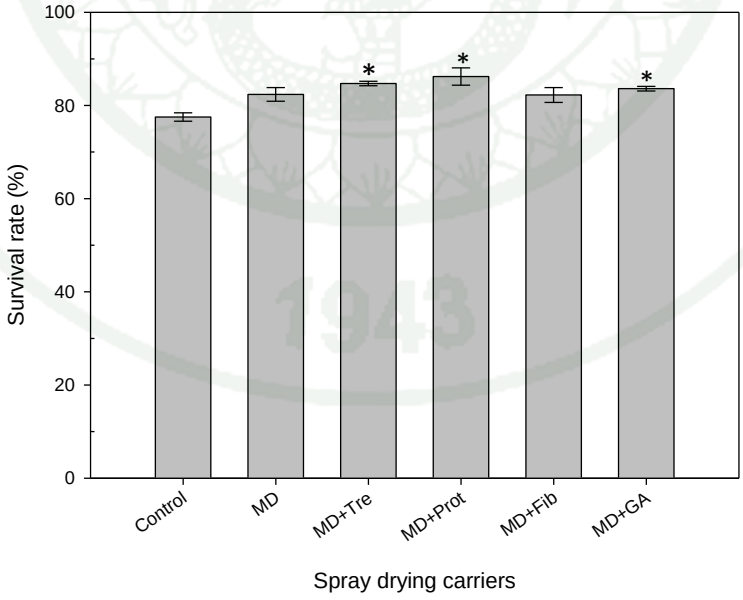

Figure 20 Survival rate of spray-dried *L. plantarum* TISTR 2075 in various carriers after exposure to small intestinal juice. Survival rates (%) of strains were compared with control, * $P < 0.05$ (Student's *t*-test, two tailed).

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Spray-dried *L. plantarum* TISTR 2075 in various carriers were also found to exhibit antimicrobial activity against *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311 with the inhibition zone ranging from 12.3 to 12.6 mm and 14.3 to 14.4 mm, respectively (Table 17). Comparing to control, it could be indicated that antimicrobial activity of *L. plantarum* TISTR 2075 was not affected by drying process. This was in agreement with Silva *et al*. (2002) that spray-dried *L. salivarius* could maintain the antimicrobial activity against *L. innocua, L. monocytogenes* and *Staphylococcus aureus.*

Table 17 Antimicrobial activity of spray-dried powders.

Spray dried carriers	Antibacterial activity against indicator strains (mm. ± S.D.)	
	E. coli O157:H7 DMST 12743	*S.* Typhimurium ATCC 13311
Control	12.0 ± 0.1	14.2 ± 0.3
MD	12.4 ± 0.5	14.3 ± 0.5
MD+Tre	12.4 ± 0.7	14.3 ± 0.5
MD+Prot	12.6 ± 0.5*	14.4 ± 0.6
MD+Fib	12.4 ± 0.5	14.3 ± 0.4
MD+GA	12.3 ± 0.3	14.3 ± 0.4

Antimicrobial activity (mm.) of strains against each pathogen was compared with control, * $P < 0.05$ (Student's *t*-test, two tailed).

6. Storage stability of spray-dried *L. plantarum* TISTR 2075

6.1 Effect of storage temperatures and protective agents on the viability of spray-dried *L. plantarum* TISTR 2075

Since the effectiveness of probiotic consumption towards human health is related to their viability, it is of utmost importance to not only minimize cell death

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during the spray drying process but also to ensure minimal loss in the viability of the dried bacteria during storage. As shown in Figure 21, it was obvious that the storage temperature was a crucial parameter affecting the survival of spray-dried cells. High storage temperatures led to a decrease in the number of viable bacteria (Foerst *et al.*, 2011). During storage at 4 °C for 12 months, it was observed that the viability of *L. plantarum* TISTR 2075 was quite stable with survival rate of 60.35–94.03% depending on protectants. At an elevated storage temperature of 25 °C, a significant ($P < 0.05$) loss of viability was observed in all protectants until reaching total destruction at 4 months. This was supported by many studies that storage temperature was a critical parameter affecting the survival of microorganisms during storage (Wang *et al.*, 2004; Zayed and Roos, 2004; Simpson *et al.*, 2005; Sorokulova *et al.*, 2008; Abe *et al.*, 2009a; Bamrungna, 2009; Chotigo, 2009; Krumnow *et al.*, 2009; Strasser *et al.*, 2009; Heidebach *et al.*, 2010; Toledo *et al.*, 2010; Ying *et al.*, 2010; Golowczyc *et al.*, 2011b). Bucio *et al*. (2005) also suggested that at low temperature, such as in a refrigerator, bacterial metabolism decreases and the accumulation of toxic wastes from the metabolism are likely minimized.

The influence of protective agents on the viability of strain during storage at different temperatures was also determined. The protective ability of protectants could be expressed in term of specific rate of degradation (k, day^{-1}). As shown in Table 18, the addition of protectants efficiently increased the storage stability of dehydrated *L. plantarum* TISTR 2075 comparing to control except the addition of Asc. MD+Tre and MD+Prot were found to be effective at both temperatures with lower k values. At 4 °C, MD+Tre and MD+Prot exhibited k values of 1.56×10^{-3} and 1.66×10^{-3} day^{-1} with the final viable cell count of 7.83 and 8.14 log CFU/g after 12 months of storage, respectively. The same tendency was also observed at non-refrigerated temperature. MD+Tre and MD+Prot showed similarly k values of 5.71×10^{-2} and 5.57×10^{-2} day^{-1} which were close to k value of *B. lactis* BB12 dried in MD+SPI ($k = 5.30 \times 10^{-2}$ day^{-1}) in previous report of Chavez *et al*. (2007). However, *L. plantarum* TISTR 2075 could maintain the viability of $> 10^6$ CFU/g for 21 days comparing to those of 90 days in *B. lactis* BB12 (Chávez and Ledeboer, 2007).

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Unfortunately, MD+Asc was found to be less effective at both temperatures with the highest *k* values of 8.17×10^{-3} and 3.10×10^{-1} day^{-1}, respectively. The final viability was 4.15 log CFU/g at 4 °C for 360 days and the totally loss of viability was observed after storage at 25 °C for 21 days. During storage, the membrane lipid oxidation, as shown by the decrease in unsaturated/saturated fatty acids ratio, seems to be a detrimental factor for cell death. Changes in the degree of unsaturation of lipids strongly affect the passive permeability of the membrane. Rapid decrease in viability tends to occur at the early storage period and change in lipid composition of the cell membrane was found to increase with time (Teixeira *et al.*, 1996; Santivarangkna *et al.*, 2007). Additionally, it is known that the consequence of many biological oxidations is the formation of free radical. The attack by free radicals on fatty acid moieties lowers the hydrophobicity owing to the introduction of hydrophilic groups and therefore weakens the hydrophobic interaction with membrane proteins, which may be essential for their activity (Santivarangkna *et al.*, 2008b).

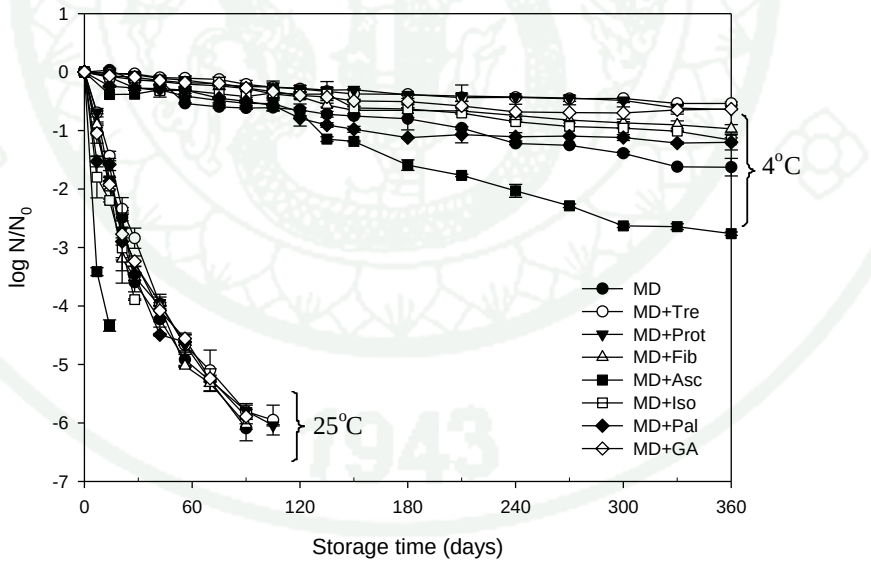

Figure 21 Relationship between storage time and temperatures (4 and 25 °C) on the survival fraction (log N/N_0) of *L. plantarum* TISTR 2075.

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Table 18 Specific rate of degradation (k, day^{-1}) of *L. plantarum* TISTR 2075 in MD with different protectants during storage at 4 and 25 °C.

Spray drying carriers	Storage temperature at 4 °C		Storage temperature at 25 °C	
	k_4 (day^{-1}) [a]	R^2 [b]	k_{25} (day^{-1}) [a]	R^2 [b]
MD	4.48×10^{-3}	0.96	6.49×10^{-2}	0.92
MD+Tre	1.56×10^{-3}	0.91	5.71×10^{-2}	0.92
MD+Prot	1.66×10^{-3}	0.96	5.57×10^{-2}	0.91
MD+Fib	2.71×10^{-3}	0.95	6.36×10^{-2}	0.90
MD+Asc	8.17×10^{-3}	0.97	3.10×10^{-1}	0.90
MD+Iso	2.90×10^{-3}	0.92	1.28×10^{-1}	0.95
MD+Pal	3.31×10^{-3}	0.85	8.21×10^{-2}	0.91
MD+GA	2.00×10^{-3}	0.89	6.16×10^{-2}	0.96

[a] the slope of the regression lines, as shown in Figure 21, were taken the inactivation rates.

[b] R^2: correlation coefficient

From these results, Prot, Tre, Fib and GA were selected for further study on the influence of relative humidity under different storage temperatures on the viability of *L. plantarum* TISTR 2075 during storage.

6.2 Effect of relative humidity on the viability of spray-dried *L. plantarum* TISTR 2075 kept under different storage temperatures

The stability of *L. plantarum* TISTR 2075 kept under different relative humidity and temperatures could be considered from k values. The k values increased continuously depending on temperatures and relative humidity (Figure 22). Also, the changes in k values depended on the type of carriers (Table 19). Storage temperature at 4 °C has a modest effect on the k values, while more deteriorative effect was observed at higher temperatures. At non-refrigerated temperatures of 25 °C, the

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lowest *k* values of *L. plantarum* TISTR 2075 were observed in JTS (1.80×10^{-2} day^{-1}) under 11% RH and MD+Tre under 23% (5.14×10^{-2} day^{-1}) and 33% RH (6.56×10^{-2} day^{-1}), respectively. The same tendency was also observed at 40 °C, dried *L. plantarum* TISTR 2075 in JTS kept under 11% RH exhibited the lowest *k* value of 6.80×10^{-2} day^{-1}, while dried *L. plantarum* in MD+Tre displayed the lowest *k* values of 1.12×10^{-1} and 1.15×10^{-1} day^{-1} under 23 and 33% RH, respectively. Unfortunately, MD+Fib and MD+GA could not effectively protect cell during storage at 40 °C. The dramatically decreased in viability of *L. plantarum* TISTR 2075 to undetectable value was observed during equilibrated under 23 and 33% RH at 40 °C for 7 days. Many studies have proved that the increasing of the relative humidity of environment at which samples were kept caused an increase in water mobility and the higher loss of viability (Castro *et al.*, 1996; King *et al.*, 1998; Achour *et al.*, 2001; Higl *et al.*, 2007; Tsen *et al.*, 2007; Tonon *et al.*, 2009; Ying *et al.*, 2010). Additionally, the low storage temperatures could slow down the adverse reactions of microorganisms, enzyme and lipid oxidation (Sablani *et al.*, 2009). This was supported by the report of Foerst *et al.* (2011) that the survival of vacuum-dried *L. paracasei* F19 were more stable under 33% RH at 20 °C than that of 37 °C. Consistent with Ying *et al.* (2010), the storage stability of *L. rhamnosus* GG encapsulated in an emulsion-based formulation stabilized by whey protein and resistant starch decreased when the samples were kept at non-refrigerated conditions and high relative humidity. From the results, survival of spray-dried *L. plantarum* TISTR 2075 did not only depend on the efficiency of spray-dried carrier protected cell after drying but also depended on the environment keeping microorganisms. This implies that the storage of probiotics at high temperature can still be possible when the relative humidity is very low. Consequently, MD+Tre and MD+Prot could be considered as good carriers at high storage temperatures (25 and 40 °C) and relative humidity of 23 and 33% RH.

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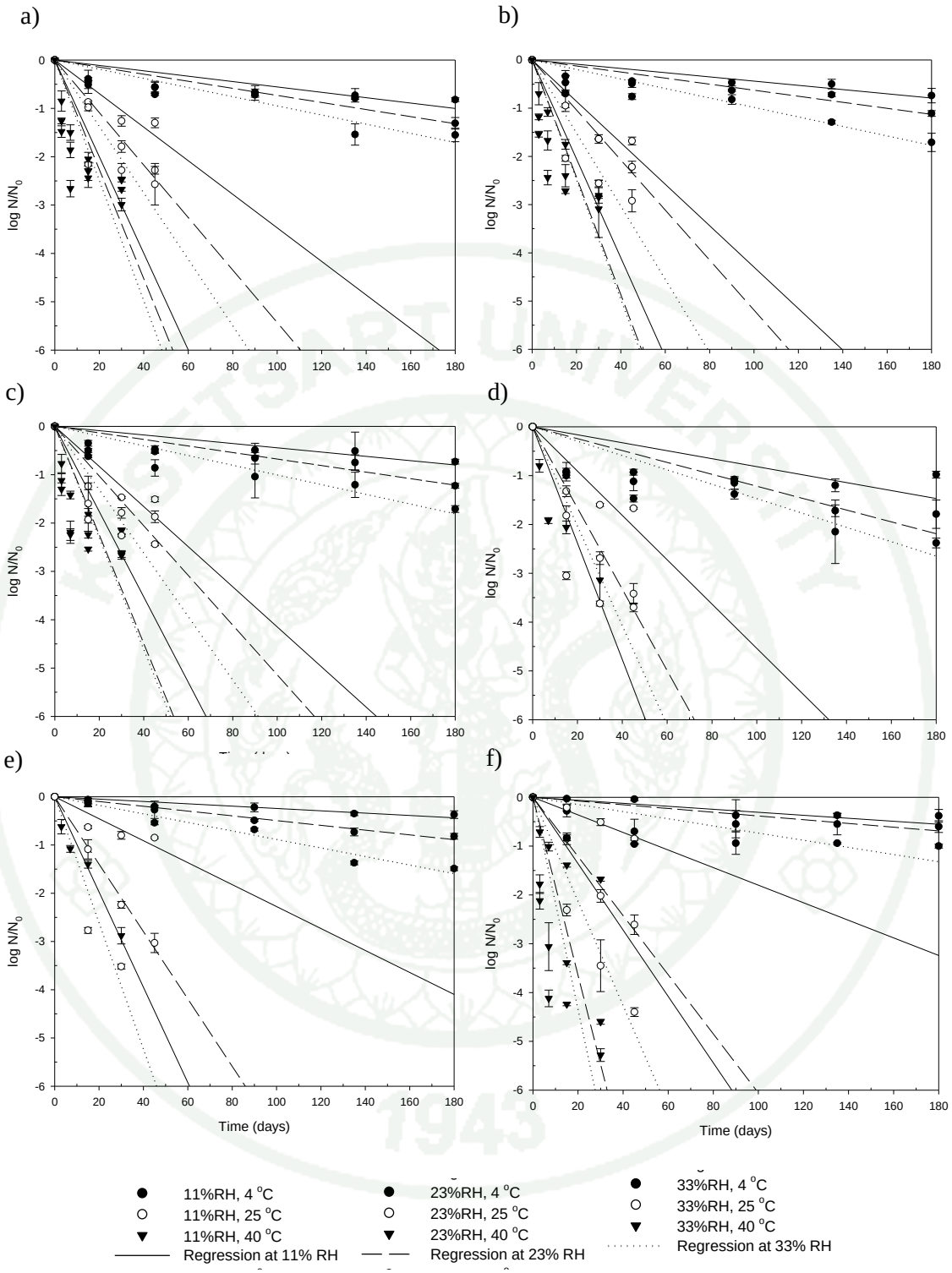

Figure 22 Storage stability of spray-dried *L. plantarum* TISTR 2075 expressed as the logarithmic values of relative survival fraction (log N/N_0) against storage time (a) MD; (b) MD+Prot; (c) MD+Tre; (d) MD+Fib; (e) MD+GA and (f) JTS.

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Table 19 Specific rate of degradation of *L. plantarum* TISTR 2075 in different spray drying carriers under various storage conditions.

Relative Humidity (%RH)	Temperature (°C)	Specific rate of degradation (k, day^{-1})					
		Protectants					
		MD	MD+Prot	MD+Tre	MD+Fib	MD+GA	JTS
11%	4	5.56×10^{-3}	4.39×10^{-3}	4.44×10^{-3}	8.16×10^{-3}	2.42×10^{-3}	3.07×10^{-3}
	25	3.47×10^{-2}	4.30×10^{-2}	4.15×10^{-2}	4.54×10^{-2}	2.28×10^{-2}	1.80×10^{-2}
	40	9.99×10^{-2}	1.03×10^{-1}	8.82×10^{-2}	1.19×10^{-1}	9.87×10^{-2}	6.80×10^{-2}
23%	4	7.33×10^{-3}	6.28×10^{-3}	6.77×10^{-3}	1.22×10^{-2}	4.94×10^{-3}	3.84×10^{-3}
	25	5.43×10^{-2}	5.19×10^{-2}	5.14×10^{-2}	8.31×10^{-2}	6.98×10^{-2}	6.06×10^{-2}
	40	1.13×10^{-1}	1.22×10^{-1}	1.12×10^{-1}	N.D.	N.D.	1.82×10^{-1}
33%	4	9.48×10^{-3}	9.85×10^{-3}	1.00×10^{-2}	1.47×10^{-2}	8.85×10^{-3}	7.33×10^{-3}
	25	6.87×10^{-2}	7.59×10^{-2}	6.56×10^{-2}	1.02×10^{-1}	1.31×10^{-1}	1.07×10^{-1}
	40	1.24×10^{-1}	1.24×10^{-1}	1.15×10^{-1}	N.D.	N.D.	2.17×10^{-1}

N.D: Not detected0

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6.3 Effect of relative humidity and temperatures on physical properties of spray-dried powders

The effect of relative humidity and temperature on physical properties of spray-dried powder during storage was evaluated in term of T_g. As shown in Table 20–22, T_g value increased with increasing temperatures at a constant relative humidity, while T_g value decreased with increasing relative humidity at a constant temperature in all spray-dried powders. For example, T_g values of MD+Tre equilibrated under 33% RH kept at 4, 25 and 40 °C was 40.23, 51.47 and 61.43 °C, respectively. This was probably because of the removal of water in samples under high storage temperatures (Bhandari and Adhikari, 2009). Due to the dependency of T_g on a_w, the stabilized T_g reflected the equilibration between sample a_w and relative humidity of the storage environment (Higl *et al.*, 2007). Water adsorption of the particles at high humidity resulted in plasticization that lowers the T_g of the particles (Shrestha *et al.*, 2007). Thus, T_g values of MD+Tre kept at 25 °C was 59.75, 54.21 and 51.47 °C equilibrated under 11, 23 and 33% RH, respectively, Furthermore, results revealed that protectants affected the T_g values of MD. Under storage at 4 °C, a significant increase ($P < 0.05$) in T_g was observed when Prot, Tre and GA were added.

It was proposed that a large difference $T–T_g$ is an indicator of stability of dried foodstuffs towards degradation processes (Palzer, 2005). Below T_g, the physical status of the material remains virtually unchanged resulting in a maximum shelf life (Bhandari and Hartel, 2005). Attempts were made to verify whether the difference in $T–T$g of drying media correlated with low specific rate of degradation (k). The results revealed that cell stored in continuous glassy matrices with a great difference in $T–T$g seem to retain their viability better than cells within matrices stored at temperatures close to glass transition (Figure 23). For example, the viability of cells stored at 4 °C was higher than at 25 and 40 °C at a constant relative humidity. A few researchers suggested that the optimal storage temperature of freeze-dried cell of *L. coryniformis* (Schoug *et al.*, 2010) and *L. rhamnosus* (Pehkonen *et al.*, 2008) should be ca. 50 and

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20–30 °C below their T_g, respectively. The glassy state does not play major protective role against storage deteriorative reactions, even all evaluated samples in this study were in the glassy state ($T_g > T$ = 13.7–44.0 °C). A wide variety of detrimental chemical reactions and physical processes may also contribute to the loss of viability in bacteria cultures during storage. The role of membrane oxidation and oxidation processes in general as well as protein-sugar reactions (Maillard reactions) should be considered. Since membrane deteriorization and compounds toxic to the cell produced during storage may be responsible for cell death (Kurtmann *et al.*, 2009b).

7. Prediction of storage stability of spray-dried *L. plantarum* TISTR 2075

Accelerated storage test is used to develop a model system to predict the long-term preservation of lactic acid bacteria (Desmond *et al.*, 1998; King *et al.*, 1998; Hamsupo *et al.*, 2005; Tsen *et al.*, 2007). The *k* values of spray-dried *L. plantarum* TISTR 2075 were obtained from the regression lines between cell viability and storage time at different temperatures (Table 23). The correlation between temperature and *k* value can be described by the Arrhenius equation as shown in equation 1.

$$k = Ae^{(-E_a/RT)} \qquad (1)$$

where *k* is the specific rate of degradation (day^{-1}), E_a is the energy of activation ($J.mole^{-1}$), R is the gas constant (8.32 $J.mole^{-1}.K^{-1}$), and T is the absolute temperature (K). When taking the logarithm of both sides of Equation 1, the Equation 2 is obtained.

$$\log k = \log k_0 - \frac{E_a}{2.303\ R} \times \frac{1}{T} \qquad (2)$$

From Arrhenius equation; constant value $\frac{Ea}{2.303\ R}$ was achieved from slope of straight line when the logarithms of determined *k* values (Table 24) were plotted against the reciprocals of their absolute temperatures as shown in Figure 24. Consequently, k_4 and k_{25} were estimated from the Arrhenius equation which allowed

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determining the energy of activation constant. The prediction equations of the strain viability for long-term preservation at 4 and 25 °C are shown in Table 24.

Arrhenius equation indicated an exponential effect of the storage temperature on the survival rate of the strain during storage. The activation energy calculated from Arrhenius equation is a good indicator to compare thermoresistance and death rate of microorganisms during spray drying, even though it is not a true value (Kim and Bhowmik, 1990). The predicted *k* value of spray-dried *L. plantarum* TISTR 2075 in various protectants was verified by the experimental *k* values. As shown in Table 18 and 24, the predicted *k* values of all protectants tested were approximately 1.8–6.7 and 1.9–3.4 times higher than the experimental *k* values at 4 and 25 °C, respectively. This was probably due to a change in physical state of spray-dried powders during accelerated storage testing which may change activation energy (Labusa and Riboh, 1982; Karmas *et al.*, 1992; Roos *et al.*, 1996). An increase in activation energy at the temperature approaching the T_g of the powders (Karmas *et al*., 1992; Roos *et al*., 1996) resulted in higher *k* values (Lapsiri *et al*., 2012b). Another possible reason why the model overestimates is a possible contribution of nonenzymatic browning. The increasing nonenzymatic browning reaction rates resulted from increasing temperatures and water content (Karmas *et al.*, 1992; Kurtmann *et al.*, 2009a; Kurtmann *et al.*, 2009b). Rate of browning was low below a critical temperature, above which the rate of the reaction increased substantially (Roos, 2001). Nonenzymatic browning is not always prevented in the glassy state. The reaction rates were much lower at temperature below T_g as compared with temperature above T_g (Kawai *et al.*, 2005).

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Table 20 Glass transition temperatures and moisture content of spray-dried *L. plantarum* TISTR 2075 with various carriers equilibrated under 11% RH at 4, 25 and 40 °C.

Carriers	T_g (°C ± S.D.)			Moisture content (% ± S.D.)		
	4 °C	25 °C	40 °C	4 °C	25 °C	40 °C
MD	44.12 ± 0.20 ^{cd}	56.24 ± 0.71 ^{c}	67.85 ± 0.88 ^{a}	6.39 ± 0.08	4.52 ± 0.08	2.89 ± 0.14
MD+Prot	46.64 ± 0.11 $^{ab/*}$	56.59 ± 0.34 ^{bc}	68.52 ± 0.25 ^{a}	6.42 ± 0.13	4.88 ± 0.25	2.51 ± 0.17
MD+Tre	46.69 ± 1.32 ^{ab}	59.75 ± 0.09 $^{a/*}$	66.29 ± 0.83 ^{b}	6.33 ± 0.24	3.23 ± 0.16	2.95 ± 0.16
MD+Fib	42.20 ± 0.97 ^{d}	54.55 ± 0.13 ^{d}	67.11 ± 0.72 ^{ab}	6.39 ± 0.14	3.67 ± 0.10	2.88 ± 0.30
MD+GA	48.00 ± 1.15 $^{a/*}$	57.54 ± 0.66 ^{b}	64.64 ± 0.12 $^{c/*}$	6.20 ± 0.17	4.37 ± 0.18	3.00 ± 0.20
JTS	45.67 ± 0.01 $^{bc/*}$	52.40 ± 0.45 $^{e/*}$	67.39 ± 0.18 ^{ab}	6.77 ± 0.18	5.46 ± 0.11	2.92 ± 0.34

Values in the same column of the same temperature tested with different lowercase letters (a–e) are significantly by Duncan's multiple range test ($P < 0.05$). T_g (°C) of various spray-dried powders equilibrated at each temperature under 11% RH was compared with MD, * $P < 0.05$ (Student's *t*-test, two tailed).

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Table 21 Glass transition temperatures and moisture content of spray-dried *L. plantarum* TISTR 2075 with various carriers equilibrated under 23% RH at 4, 25 and 40 °C.

Carriers	T_g (°C ± S.D.)			Moisture content (% ± S.D.)		
	4 °C	25 °C	40 °C	4 °C	25 °C	40 °C
MD	41.56 ± 0.00 ^{c}	54.31 ± 0.37 ^{b}	67.13 ± 0.08 ^{a}	6.67 ± 0.13	3.45 ± 0.01	3.02 ± 0.03
MD+Prot	44.61 ± 0.46 $^{ab/*}$	55.20 ± 0.56 ^{b}	67.66 ±0.71 ^{a}	6.48 ± 0.10	4.91 ± 0.13	2.63 ± 0.07
MD+Tre	45.33 ± 0.25 $^{a/*}$	54.21 ± 0.72 ^{b}	62.70 ± 0.40 $^{b/*}$	6.34 ± 0.08	3.26 ± 0.17	3.22 ± 0.13
MD+Fib	41.08 ±1.27 ^{c}	52.77 ± 0.78 ^{c}	66.90 ± 0.09 ^{a}	7.58 ± 0.16	5.50 ± 0.10	3.05 ± 0.23
MD+GA	43.44 ± 0.46 $^{b/*}$	57.05 ± 0.44 $^{a/*}$	63.20 ± 0.29 $^{b/*}$	6.80 ±0.06	4.47 ± 0.04	3.29 ± 0.14
JTS	44.00 ± 0.91 ^{ab}	51.31 ± 0.06 $^{d/*}$	67.10 ± 0.04 ^{a}	6.67 ±0.17	5.60 ± 0.14	2.93 ± 0.16

Values in the same column of the same temperature tested with different lowercase letters (a–e) are significantly by Duncan's multiple range test ($P < 0.05$). T_g (°C) of various spray-dried powders equilibrated at each temperature under 23% RH was compared with MD, * $P < 0.05$ (Student's *t*-test, two tailed).

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Table 22 Glass transition temperatures and moisture content of spray-dried *L. plantarum* TISTR 2075 with various carriers equilibrated under 33% RH at 4, 25 and 40 °C.

Carriers	T_g (°C ± S.D.)			Moisture content (% ± S.D.)		
	4 °C	25 °C	40 °C	4 °C	25 °C	40 °C
MD	36.31 ± 0.09 ^{c}	51.88 ± 0.43 ^{b}	64.94 ± 1.44 ^{a}	9.79 ± 0.20	5.44 ± 0.14	3.05 ± 0.08
MD+Prot	38.54 ± 0.01 $^{b/*}$	51.77 ± 0.19 ^{b}	65.10 ± 0.85 ^{a}	9.81 ± 0.17	5.42 ± 0.16	3.10 ± 0.11
MD+Tre	40.23 ± 0.27 $^{ab/*}$	51.47 ± 0.18 ^{b}	61.43 ± 1.27 ^{b}	7.55 ± 0.07	5.40 ± 0.13	3.40 ± 0.14
MD+Fib	40.31 ± 0.93 $^{ab/*}$	51.74 ± 0.59 ^{b}	62.02 ± 0.24 ^{b}	9.07 ± 0.13	5.52 ± 0.06	3.36 ± 0.07
MD+GA	41.96 ± 1.00 $^{a/*}$	55.79 ± 1.04 $^{a/*}$	60.00 ± 0.33 $^{b/*}$	7.43 ± 0.10	4.53 ± 0.14	3.50 ± 0.16
JTS	39.30 ± 1.14 ^{b}	46.10 ± 0.37 $^{c/*}$	53.66 ± 0.25 $^{c/*}$	8.55 ± 0.18	6.03 ± 0.11	5.23 ± 0.13

Values in the same column of the same temperature tested with different lowercase letters (a–e) are significantly by Duncan's multiple range test ($P < 0.05$). T_g (°C) of various spray-dried powders equilibrated at each temperature under 33% RH was compared with MD, * $P < 0.05$ (Student's *t*-test, two tailed).

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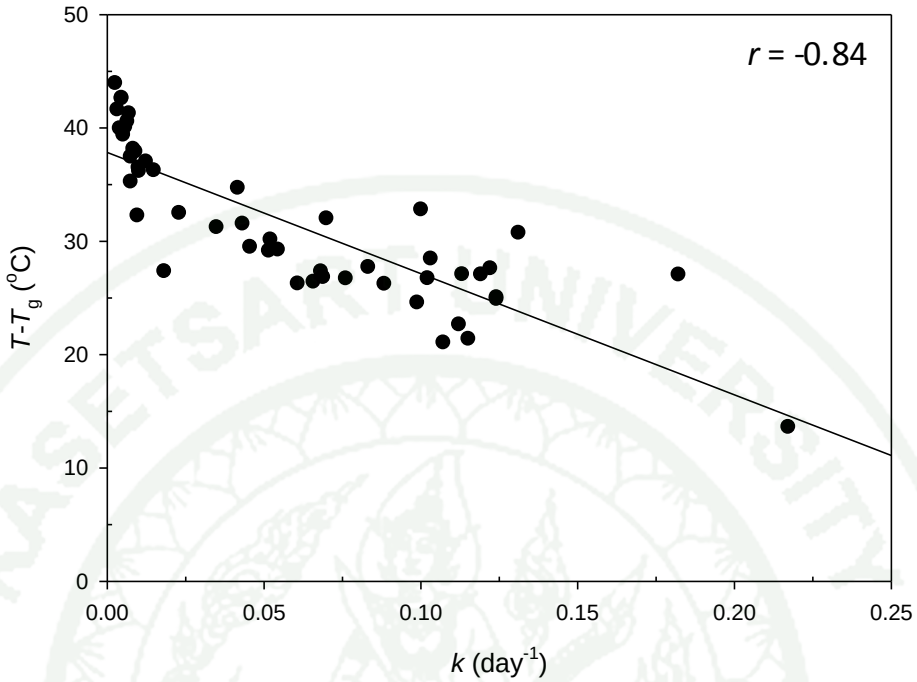

Figure 23 Correlation between the difference in $T–T_g$ and the specific rate of degradation (k, day^{-1}).

Table 23 Specific rate of degradation of *L. plantarum* TISTR 2075 at accelerated temperatures of 37, 45, 60 and 80 °C.

Spray-dried carriers	Specific rate of degradation (k, day^{-1}) (R^2) at different temperatures			
	37 °C	45 °C	60 °C	80 °C
MD	0.71 (0.946)	0.13 (0.988)	6.67 (0.970)	52.82 (0.802)
MD+Tre	0.67 (0.990)	1.05 (0.979)	6.22 (0.977)	53.06 (0.929)
MD+Prot	0.71 (0.999)	1.12 (0.972)	5.73 (0.995)	52.80 (0.999)
MD+Fib	0.70 (0.982)	1.36 (0.968)	6.27 (0.994)	59.38 (0.984)
MD+Iso	1.17 (0.969)	1.68 (0.878)	6.82 (0.956)	53.40 (0.811)
MD+Pal	0.80 (0.934)	1.27 (0.821)	5.76 (0.989)	40.32 (0.967)
MD+GA	0.67 (0.944)	1.31 (0.921)	7.38 (0.983)	53.90 (0.951)

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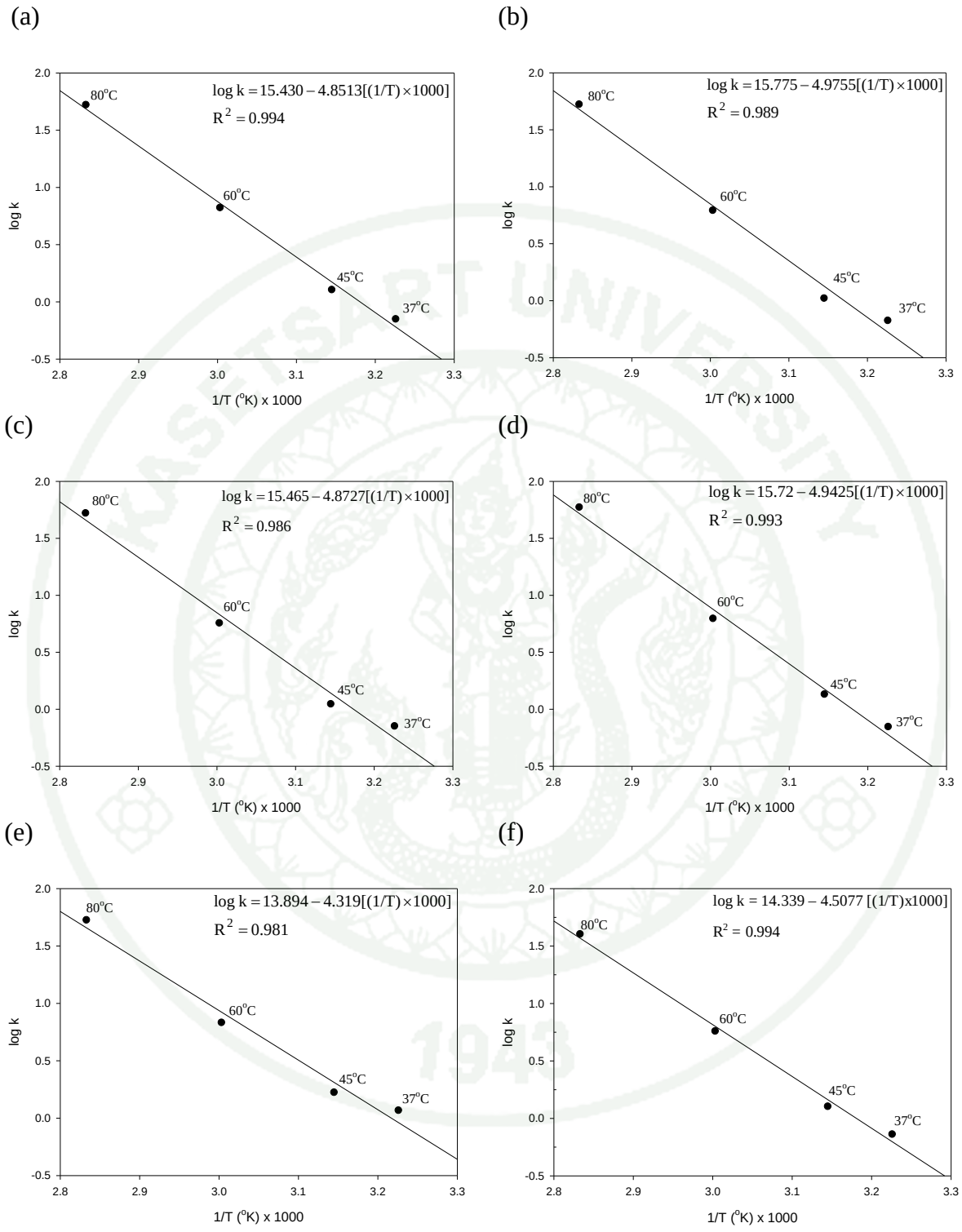

Figure 24 Arrhenius plots of the specific rate of degradation (*k*) of spray-dried *L. plantarum* TISTR 2075 at different temperatures in various protectants (a) MD; (b) MD+Tre; (c) MD+Prot; (d) MD+Fib; (e) MD+Iso; (f) MD+Pal and (g) MD+GA.

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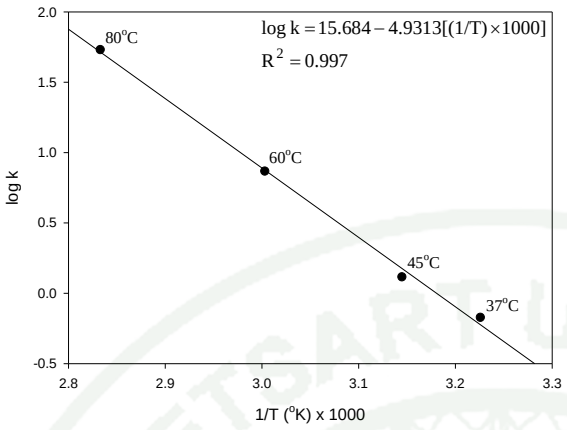

(g)

Figure 24 (Continued)

Table 24 Predicted specific rate of degradation of *L. plantarum* TISTR 2075 in different protectants during storage at 4 and 25 °C.

Spray drying protectants	Predicted values k (day^{-1})	
	k_4	k_{25}
MD (control)	8.25×10^{-3}	1.41×10^{-1}
MD+Tre	6.50×10^{-3}	1.12×10^{-1}
MD+Prot	7.48×10^{-3}	1.30×10^{-1}
MD+Fib	7.53×10^{-3}	1.36×10^{-1}
MD+Iso	2.00×10^{-2}	4.36×10^{-1}
MD+Pal	1.16×10^{-2}	1.63×10^{-1}
MD+GA	7.61×10^{-3}	1.37×10^{-1}

Additionally, Labuza and Riboh (1982) revealed several possible reasons for deviations from the Arrhenius kinetics: (i) an increase in temperature may cause the occurrence of first-order phase transitions, e.g. melting of solid fat which may increase mobility of potential reactants in the resultant liquid phase; (ii) reactions with different activation energies may predominate at different temperatures; (iii) loss of

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water at high temperatures may alter reaction rates; (iv) proteins at high temperatures may become more or less susceptible to chemical reactions due to denaturation; (iv) increasing water activity with increasing temperature may cause an additional increase in reaction rate; and (v) crystallization of amorphous sugars may release water and affect the proportion of reactants in the solute-water phase.

Although, these predicted models deviated from the Arrhenius theory, few studies have been successful to predict the viability of microorganism during storage. According to Wirunpan (2011), there was no great difference between predicted and experimental *k* values of *L. lactis* 1464 viability of the strain in feed pellet with addition of MSG and milk powder during storage at 4 °C over 12 months. However, at storage temperature of 30 °C, the experimental *k* value was approximately 1.4 and 2.2 times higher than predicted *k* value of the strain with addition of MSG and milk powder, respectively. Hamsupo *et al*. (2005) reported that there was no significant difference in viability between prediction and experimental survival rates of spray-dried *L. reuteri* KUB-AC5 at 4 and 30°C for 4 months. This indicated that the predicted model may probably vary according to the strain of microorganisms and also the capacity of the protectants used. Therefore, accelerated storage testing was found to be a simple technique but with only certain degree of correctness and predictability.

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CONCLUSION

In the present study, *L. plantarum* TISTR 2075 isolated from fermented vegetables showed above-average results in all criteria observed in vitro and could be considered as potential probiotic. This strain showed strong autoaggregation and cell surface hydrophobicity which is related to the adhesion ability to intestinal cells. The strain had positive coaggregation with *E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311 linked to the ability to interact closely with these pathogens and it also had antimicrobial activity against both pathogens. In addition, the strain was resistant to some antibiotics used in human clinical therapy, especially vancomycin. The ability to survive under gastrointestinal tract conditions was also observed. Furthermore, the strain was able to withstand a high temperature of 65 °C for 30 min which is a technological criterion in commercial application. This would guarantee that the strain will maintain the viability during the drying process required for prolonged storage.

It was important to find out that cereal flour have the capability to protect all test strains during exposure to gastrointestinal tract conditions. They exhibited the ability to tolerate simulated gastrointestinal tract conditions and their tolerances to acid and bile juices were greatly enhanced by the addition of soybean, sesame and Job's Tears flour. The protective capacity of the cereals was dependant on their compositions. Soybean was found to enhance the acid tolerance of the strains while Job's Tears improved the bile tolerance. These results suggest that the cereal flour could be used as vehicle for the delivery of probiotic lactic acid bacteria through the human gastrointestinal tract. Additionally, soymilk and Job's Tears extracts supplemented with sesame could be used as culture media for the growth of *L. plantarum* TISTR 2075 with the viable cell count maintained above 8.3 log CFU/mL. The significant increase in calcium solubility was also observed after fermentation related to lowered pH associated with production of lactic acid. This finding could further lead to the development of new synbiotic food products containing probiotic and prebiotic cereals.

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L. plantarum TISTR 2075 has the ability to survive during spray drying process depending on the type of protectants. Protein and trehalose were found to provide high viable cell number after spray drying with the viable cell number of 8.8 and 8.4 log CFU/g, respectively, which meets the minimum recommended levels (6–7 log CFU/mg) of probiotics in foods. After spray drying, the functional properties of probiotic including antimicrobial activity against foodborne pathogens (*E. coli* O157:H7 DMST 12743 and *S.* Typhimurium ATCC 13311) and the tolerance to simulated gastrointestinal tract conditions were not affected. The results revealed that spray drying could produce stable powders of *L. plantarum* TISTR 2075 with high viable cell number.

The storage temperature and relative humidity influenced the viability of spray-dried *L. plantarum* TISTR 2075 and physical property of spray-dried powders. Higher storage temperature induced higher specific rate of degradation of spray-dried *L. plantarum* TISTR 2075. Also, increasing the relative humidity resulted in higher loss of viability. From a commercial point of view, probiotic products should actually be stored at non-refrigerated temperatures. The results revealed that maltodextrin with the addition of trehalose or protein could be considered as good carrier for storage under 23 and 33% RH at 25 °C. Furthermore, storage conditions were found to affect the physical state in terms of glass transition temperature (T_g) of spray-dried powders. The T_g value increased with increasing temperatures at a constant relative humidity, while T_g value decreased with increasing relative humidity at a constant temperature. In order to avoid the dehydrated structure changes, powders should be stored below their T_g. Besides, inactivation rates are certainly lower in the glassy state.

The prediction models based on Arrhenius theory for estimating stability of *L. plantarum* TISTR 2075 in various carriers during storage were developed by using accelerated storage test. The kinetic analyses of accelerated storage test data induced the equation indicating the prediction model of the strain survival during storage. These models could be extrapolated the strain viability stored at 4 and 25 °C with only certain correctness and predictability. However, it must require the correction

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factor to rectify the models. These predictive equations will be useful for probiotics feed manufacturers to design and expect the probiotic shelf-life.

With regards to developing this research into the future, it would be worthwhile to investigate the large scale production of probiotic fermented cereal extracts and vegetable products in order to assess the capability of providing cost-effective and industrial-scale quantities for commercial. Further investigations should be a confirmation of the efficiency of probiotic through conducted intervention clinical dietary trials to assure the potential health benefits to the consumers. The consumer acceptability should be also evaluated.

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LITERATURE CITED

Abe, F., H. Miyauchi, A. Uchijima, T. Yaeshima and K. Iwatsuki. 2009. Effects of storage temperature and water activity on the survival of *Bifidobacteria* in powder form. **Int. J. Dairy Technol.** 62 (2): 234–239.

Achour, M., N. Mtimet, C. Cornelius, S. Zgouli, A. Mahjoub, P. Thonart and M. Hamdi. 2001. Application of the accelerated shelf life testing method (ASLT) to study the survival rates of freeze-dried *Lactococcus* starter cultures. **J. Chem. Technol. Biotechnol.** 76: 624–628.

Adhikari, B., T. Howes, B.R. Bhandari and V. Truong. 2003. Characterization of the surface stickiness of fructose-maltodextrin solutions during drying. **Dry. Technol.** 21 (1): 17–34.

Agarwal, K.N. and S.K. Bhasin. 2002. Feasibility studies to control acute diarrhoea in children by feeding fermented milk preparations actimel and Indian Dahi. . **Eur. J. Clin. Nutr.** 56 (suppl 4): S56–S59.

Ammor, S., G. Tauveron, E. Dufour and I. Chevallier. 2006. Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility 1-screening and characterization of the antibacterial compounds. **Food Control** 17: 454–461.

Ananta, E. 2005. **Impact of environmental factors on viability and stability and high pressure pretreatment on stress tolerance of *Lactobacillus rhamnosus* GG (ATCC 53103) during spray drying.** Dr. Ing., Prozesswissenschaften der Technischen Universität Berlin.

Ananta, E., M. Volkert and D. Knorr. 2005. Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. **Int. Dairy J.** 15 (4): 399–409.

Copyright by Kasetsart University All rights reserved

Angelov, A., V. Gotcheva, R. Kuncheva and T. Hristozova. 2006. Development of a new oat-based probiotic drink. **Int. J. Food Microbiol.** 112: 75–80.

AOAC. 1990. **Official methods of analysis of AOAC** 15th ed. Association of Official Analytical Chemists (AOAC), Arlington, VA, USA.

AOAC. 2005. **Official methods of analysis of AOC international.** 18th ed. Association of Official Analytical Chemists (AOAC), Gaithersburg, Maryland, USA.

Baek, Y.J. and B.H. Lee. 2009. Probiotics and prebiotics as bioactive components in dairy products, pp. 287–310. *In* Y. W. Park, eds. **Bioactive components in milk and dairy products.** Wiley-Blackwell, Singapore.

Bamrungna, P. 2009. **Production of fermented soymilk powder containing lactic acid bacteria by spray drying.** M.S. Thesis, Kasetsart University.

Bao, Y., Y. Zhang, Y. Zhang, Y. Liu, S. Wang, X. Dong, Y. Wang and H. Zhang. 2010. Screening of potential probiotic properties of *Lactobacillus fermentum* isolated from traditional dairy products. **Food Control** 21 (5): 695–701.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck. 1966. Antibiotic susceptibility testing by a standard single disc method. **Am. J. Clin. Pathol.** 45: 493–496.

Begley, M., C. Hill and C.G.M. Gahan. 2006. Bile salt hydrolase activity in probiotics. **Appl. Environ. Microbiol.** Mar: 1729–1738.

Bergqvist, S.W., A.S. Sandberg, N.G. Carlsson and T. Andlid. 2005. Improved iron solubility in carrot juice fermented by homo- and hetero-fermentative lactic acid bacteria. **Food Microbiol.** 22 (1): 53–61.

Copyright by Kasetsart University All rights reserved

Bhandari, B. 2008. Spray drying and powder properties, pp. 215–248. *In* Y. H. Hui, C. Clary, M. M. Farid, O. O. Fasina, A. Noomhorm and J. Welti-Chanes, eds. **Food drying science and technology: Microbiology, chemistry, application.** DEStech Publications, Inc., U.S.A.

Bhandari, B. and R.W. Hartel. 2005. Phase transition during food powder production and powder stability, pp. 262-292. *In* C. Onwulata, eds. **Encapsulated and powdered food.** Taylor & Francis, New York.

Bhandari, B. and B. Adhikari. 2009. Glass-transition based approach in drying of foods, pp. 38-62. *In* C. Ratti, eds. **Advances in food dehydration.** CRC Press, London.

Bhandari, B., K.C. Patel and X.D. Chen. 2008. Spray dring of food materials - process and product characteristics, pp. 113–159. *In* X. D. Chen and A. S. Mujumdar, eds. **Drying technologies in food processing.** Blackwell Publishing Ltd., UK.

Bhandari, B.R. and T. Howes. 1999. Implication of glass transition for the drying and stability of dried foods. **J. Food Eng.** 40 (1–2): 71–79.

Bhandari, B.R., A. Senoussi, E.D. Dumoulin and A. Lebert. 1993. Spray drying of concentrated fruit juices **Dry. Technol.** 11 (5): 1081–1092.

Bielecka, M. and A. Majkowska. 2000. Effect of spray drying temperature of yoghurt on the survival of starter cultures, moisture content and sensoric properties of yoghurt powder. **Food Nahrung** 44 (4): 257–260.

Blandino, A., M.E. Al-Aseeri, S.S. Pandiella, D. Cantero and C. Webb. 2003. Cereal-based fermented foods and beverages. **Food Res. Int.** 36: 527–543.

Copyright by Kasetsart University All rights reserved

Boonyai, P., T. Howes and B. Bhandari. 2007. Instrumentation and testing of a thermal mechanical compression test for glass-rubber transition analysis of food powders. **J. Food Eng.** 78 (4): 1333–1342.

Bozoglu, T.F., M. Ozilgen and U. Bakir. 1987. Survival kinetics of lactic acid starter cultures during and after freeze drying. **Enzym. Microb. Technol.** 9: 531–537.

Bucio, A., R. Hartemink, J.W. Schrama, J. Verreth and F.M. Rombouts. 2005. Survival of *Lactobacillus plantarum* 44a after spraying and drying in feed and during exposure to gastrointestinal tract fluids *in vitro*. **J. Gen. Appl. Microbiol.** 51: 221–227.

Buntin, N., S. Chanthachum and T. Hongpattarakere. 2008. Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. **Songklanakarin J. Sci. Technol.** 30 (Suppl.1): 141–148.

Buriti, F.C.A., I.A. Castro and S.M.I. Saad. 2010. Viability of *Lactobacillus acidophilus* in synbiotic guava mousses and its survival under *in vitro* simulated gastrointestinal conditions. **Int. J. Food Microbiol.** 137 (2–3): 121–129.

Burns, P., G. Vinderola, A. Binetti, A. Quiberoni, C.G. de los Reyes-Gavilán and J. Reinheimer. 2008. Bile-resistant derivatives obtained from non-intestinal dairy *Lactobacilli*. **Int. Dairy J.** 18 (4): 377–385.

Carvalho, A.S., J. Silva, P. Ho, P. Teixeira, F.X. Malcata and P. Gibbs. 2004. Relevant factors for the preparation of freeze-dried lactic acid bacteria. **Int. Dairy J.** 14 (10): 835–847.

Copyright by Kasetsart University All rights reserved

Castro, H.P., P.M. Teixeira and R. Kirby. 1995. Storage of lyophilized cultures of *Lactobacillus bulgaricus* under different relative humidities and atmospheres. **Appl. Microbiol. Biotechnol.** 44: 172–176.

Castro, H.P., P.M. Teixeira and R. Kirby. 1996. Changes in the cell membrane of *Lactobacillus bulgaricus* during storage following freeze-drying. **Biotechnol. Lett.** 18: 99–104.

Champagne, C.P., N.J. Gardner and D. Roy. 2005. Challenges in the addition of probiotic cultures to foods. **Crit. Rev. Food Sci. Nutr.** 45: 61–84.

Champagne, C.P., N.J. Gardner, E. Brochu and Y. Beaulieu. 1991. The freeze drying of lactic acid bacteria. a review. **Can. Inst. Sci. Technol. J.** 24: 118–128.

Chang, Y.C. and M.G. Nair. 1995. Metabolism of daidzein and genistein by intestinal bacteria. **J. Nat. Prod.** 58: 1892–1896.

Chapa, V.A. 2006. **Mimicking anhydrobiosis on solid support lipid bilayers.** M.S. Thesis, Texas A&M University.

Charalampopoulos, D., S.S. Pandiella and C. Webb. 2003. Evaluation of the effect of malt, wheat and barley extracts on the viability of potentially probiotic lactic acid bacteria under acidic conditions. **Int. J. Food Microbiol.** 82 (2): 133–141.

Charalampopoulos, D., R. Wang, S.S. Pandiella and C. Webb. 2002. Application of cereals and cereal components in functional foods: a review. **Int. J. Food Microbiol.** 79 (1–2): 131–141.

Copyright by Kasetsart University All rights reserved

Charteris, Kelly, Morelli and Collins. 1998. Antibiotic susceptibility of potentially probiotic *Bifidobacterium* isolates from the human gastrointestinal tract. **Lett. Appl. Microbiol.** 26 (5): 333–337.

Chávez, B.E. and A.M. Ledeboer. 2007. Drying of probiotics: optimization of formulation and process to enhance storage survival. **Dry. Technol.** 25 (7): 1193–1201.

Cheng, I.C., H.F. Shang, T.F. Lin, T.H. Wang, H.S. Lin and S.H. Lin. 2005. Effect of fermented soy milk on the intestinal bacterial ecosystem. **World J. Gastroenterol.** 11 (8): 1225–1227.

Chotigo, A. 2009. **Production of pelleted feed containing *Lactobacillus reuteri* KUB-AC5 by cold pelleting process.** M.S. Thesis, Kasetsart University.

Chou, C.-C. and J.-W. Hou. 2000. Growth of *Bifidobacteria* in soymilk and their survival in the fermented soymilk drink during storage. **Int. J. Food Microbiol.** 56: 113–121.

Cleveland, J., T.J. Montville, I.F. Nes and M.L. Chikindas. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. **Int. J. Food Microbiol.** 71: 1–20.

Collado, M.C., I. Surono, J. Meriluoto and S. Salminen. 2007. Indigenous dadih lactic acid bacteria: cell-surface properties and interactions with pathogens. **J. Food Sci.** 72 (3): M89–M93.

Corcoran, B., C. Stanton, S. Miao, G. Fitzgerald and P.R. Ross. 2006. Viability of probiotic bacteria as affected by drying, pp 233–247. *In* S. Catherine, F. Gerald, M. Song, R. Paul and C. Barry, eds. **Water properties of food, pharmaceutical, and biological materials.** CRC Press, New York.

Copyright by Kasetsart University All rights reserved

Corcoran, B.M., R.P. Ross, G.F. Fitzgerald and C. Stanton. 2004. Comparative survival of probiotic *Lactobacilli* spray-dried in the presence of prebiotic substances. **J. Appl. Microbiol.** 96 (5): 1024–1039.

Corcoran, B.M., C. Stanton, G.F. Fitzgerald and R.P. Ross. 2005. Survival of probiotic *Lactobacilli* in acidic environments is enhanced in the presence of metabolizable sugars. **Appl. Environ. Microbiol.** 71 (6): 3060–3067.

Cotter, P.D. and C. Hill. 2003. Surviving the acid test: responses of gram-positive bacteria to low pH. **Microbiol. Mol. Biol. Rev.** 67 (3): 429–453.

Crowe, J.H., F.A. Hoekstra and L.M. Crowe. 1992. Anhydrobiosis. **Annu. Rev. Physiol.** 54: 579–599.

Crowe, J.H., L.M. Crowe, A.E. Oliver, N. Tsvetkova, W. Wolkers and F. Tablin. 2001. The trehalose myth revisited: introduction to a symposium on stabilization of cells in the dry state. **Cryobiol.** 43 (2): 89–105.

Crowe, L.M., D.S. Reid and J.H. Crowe. 1996. Is trehalose special for preserving dry biomaterials? **Biophys. J.** 71 (4): 2087–2093.

Cruz, A.G., E.H.M. Walter, R.S. Cadena, J.A.F. Faria, H.M.A. Bolini, H.P. Pinheiro and A.S. Sant'Ana. 2010. Survival analysis methodology to predict the shelf-life of probiotic flavored yogurt. **Food Res. Int.** 43 (5): 1444–1448.

Danielsen, M. and A. Wind. 2003. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. **Int. J. Food Microbiol.** 82 (1): 1–11.

De Angelis, M. and M. Gobbetti. 2004. Environmental stress responses in *Lactobacillus*: a review. **Proteomics** 4 (1): 106–122.

Copyright by Kasetsart University All rights reserved

de Vrese, M. and J. Schrezenmeir. 2008. Probiotics, prebiotics, and synbiotics, pp. 1–66. *In* U. Stahl, U. Donalies and E. Nevoigt, eds. **Food Biotechnology.** Springer Berlin, Heidelberg.

De Vuyst, L. and F. Leroy. 2007. Bacteriocins from lactic acid bacteria: production, purification, and food applications. **J. Mol. Microbiol. Biotechnol.** 13: 194–199.

Del Piano, M., L. Morelli, G.P. Strozzi, S. Allesina, M. Barba, F. Deidda, P. Lorenzini, M. Ballare, F. Montino, M. Orsello, M. Sartori, E. Garello, S. Carmagnola, M. Pagliarulo and L. Capurso. 2006. Probiotics:from research to consumer. **Dig. Liver Dis.** 38 (Suppl. 2): S248–S255.

Del Re, B., B. Sgorbati, M. Miglioli and D. Palenzona. 2000. Adhesion, autoaggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. **Lett. Appl. Microbiol.** 31: 438–442.

DePaz, R.A., D.A. Dale, C.C. Barnett, J.F. Carpenter, A.L. Gaertner and T.W. Randolph. 2002. Effects of drying methods and additives on the structure, function and storage stability of subtilisin: role of protein conformation and molecular mobility. **Enzym. Microb. Technol.** 31 (6): 765–774.

Desmond, C., C. Stanton, G.F. Fitzgerald, K. Collins and R. Paul Ross. 2002. Environmental adaptation of probiotic *Lactobacilli* towards improvement of performance during spray drying. **Int. Dairy J.** 12 (2–3): 183–190.

Desmond, S., H. Krhouz, P. Evrard and P. Thonart. 1998. Improvement of lactic cell production. **Appl. Biochem. Biotechnol.** 70–72 (1): 513–526.

Ding, W.K. and N.P. Shah. 2007. Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. **J. Food Sci.** 72 (9): M446–M450.

Copyright by Kasetsart University All rights reserved

do Espírito Santo, A.P., P. Perego, A. Converti and M.N. Oliveira. 2011. Influence of food matrices on probiotic viability - a review focusing on the fruity bases. **Trends Food Sci. Technol.** 22 (7): 377–385.

Domrongpokkaphan, V. and P. Wanchaitanawong. 2006. In vitro antimicrobial activity of *Bacillus* spp. against pathogenic *vibrio* spp. in black tiger shrimp (*penaeus monodon*). **Kasetsart J. (Nat. Sci.).** 40: 949–957.

Donsi, F., G. Ferrari, E. Senza and P. maresca. 2009. Main factors regulating microbial inactivation by high-pressure homogenization: operating parameters and scale of operation. **Chem. Eng. Sci.** 64: 520–532.

du Toit, M., C.M.A.P. Franz, L.M.T. Dicks, U. Schillinger, P. Haberer, B. Warlies, F. Ahrens and W.H. Holzapfel. 1998. Characterisation and selection of probiotic *Lactobacilli* for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. **Int. J. Food Microbiol.** 40 (1–2): 93–104.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. **Anal. Chem.** 28: 350–356.

Dunne, C., L. O' Mahany, L. Murphy, G. Thornton, D. Morrissey, S. O' Halloran, M. Freeny, S. Flynn, G. Fitzgerald, C. Daly, B. Kiely, G. C O' Sullivan, F. Shanahan and J.K. Collins. 2001. *In vitro* selection criteria for probiotic bacteria of human origin correlation with *in vivo* findings. **Am. J. Clin. Nutr.** 73(suppl): 386S–392S.

Edema, M.O. and A.I. Sanni. 2008. Functional properties of selected starter cultures for sour maize bread. **Food Microbiol.** 25 (4): 616–625.

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Elisha, B.G. and P. courvalin. 1995. Aanlysis of genes encoding d-alanine: D-alanine ligase-related enzymes in *Leuconostoc mesenteroides* and *Lactobacillus* spp. **Gene** 152: 79–83.

El-Naggar, M.Y.M. 2004. Comparative study of probiotic cultrues to control the growth of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. **Biotechnol.** 3 (2): 178–180.

FAO/WHO. 2001. **Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria.** Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report. Cordoba, Argentina.

Farnworth, E.R. and C. Champagne. 2010. Production of probiotic cultures and their incorporation into foods, pp. 3–17. *In* W. Ronald Ross and R. P. Victor, eds. **Bioactive foods in promoting health.** Academic Press, Boston.

Foerst, P., U. Kulozik, M. Schmitt, S. Bauer and C. Santivarangkna. 2012. Storage stability of vacuum-dried probiotic bacterium *Lactobacillus paracasei* F19. **Food Bioprod. Process.** 90 (2): 295–300.

Fonseca, F., C. Beal and G. Corrieu. 2000. Method of quantifying the loss of acidification activity of lactic acid starters during freezing and frozen storage. **J. Dairy Res.** 67 (1): 83–90.

Fonseca, F., S. Passot, O. Cunin and M. Marin. 2004. Collapse temperature of freeze-dried *Lactobacillus bulgaricus* suspensions and protective media. **Biotechnol. Prog.** 20 (1): 229–238.

Fooks, L.J., R. Fuller and G.R. Gibson. 1999. Prebiotics, probiotics and human gut microbiology. **Int. Dairy J.** 9: 53–61.

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Fu, N. and X.D. Chen. 2011. Towards a maximal cell survival in convective thermal drying processes. **Food Res. Int.** 45 (5): 1127–1149.

Gardiner, G.E., E. O'Sullivan, J. Kelly, M.A.E. Auty, G.F. Fitzgerald, J.K. Collins, R.P. Ross and C. Stanton. 2000. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. **Appl. Environ. Microbiol.** 66 (6): 2605–2612.

Gbassi, G.K., T. Vandamme, S. Ennahar and E. Marchioni. 2009. Microencapsulation of *Lactobacillus plantarum* spp. in an alginate matrix coated with whey proteins. **Int. J. Food Microbiol.** 129 (1): 103–105.

Gharsallaoui, A., G. Roudaut, O. Chambin, A. Voilley and R. Saurel. 2007. Applications of spray-drying in microencapsulation of food ingredients: an overview. **Food Res. Int.** 40 (9): 1107–1121.

Giraffa, G., N. Chanishvili and Y. Widyastuti. 2010. Importance of *Lactobacilli* in food and feed biotechnology. **Res. in Microbiol.** 161 (6): 480–487.

Gobbetti, M., R.D. Cagno and M. De Angelis. 2010. Functional microorganisms for functional food quality. **Crit. Rev. Food Sci. Nutr.** 50 (8): 716–727.

Golowczyc, M.A., C.L. Gerez, J. Silva, A.G. Abraham, G.L. Antoni and P. Teixeira. 2011a. Survival of spray-dried *Lactobacillus kefir* is affected by different protectants and storage conditions. **Biotechnol. Lett.** 33 (4): 681–686.

_____, J. Silva, A.G. Abraham, G.L. De Antoni and P. Teixeira. 2010a. Preservation of probiotic strains isolated from kefir by spray drying. **Lett. Appl. Microbiol.** 50 (1): 7–12.

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Golowczyc, M.A., P. Teixeira, G.L. De Antoni and A.G. Abraham. 2011b. Cellular injuries of spray-dried *Lactobacillus* spp. Isolated from kefir and their impact on probiotic properties. **Int. J. Food Microbiol.** 144 (3): 556–560.

Gómez Zavaglia, A., E. Tymczyszyn, G. De Antoni and E. Aníbal Disalvo. 2003. Action of trehalose on the preservation of *Lactobacillus delbrueckii* ssp. *bulgaricus* by heat and osmotic dehydration. **J. Appl. Microbiol.** 95 (6): 1315–1320.

Gould, G.W. 1989. Heat-induced injury and inactivation., pp. 11–42. *In* G. W. Gould, eds. **Mechanisms of action of food preservation procedure.** Elsevier Applied Science, New York.

Granato, D., G.F. Branco, F. Nazzaro, A.G. Cruz and J.A.F. Faria. 2010. Functional foods and nondairy probiotic food development: trends, concepts, and products. **Compr. Rev. Food Sci. Food Saf.** 9 (3): 292–302.

Guzzo, J., F. Delmas, F. Pierre, M.P. Jobin, B. Samyn, J. Van Beeumen, J.F. Cavin and C. Diviès. 1997. A small heat shock protein from *Leuconostoc oenos* induced by multiple stresses and during stationary growth phase. **Lett. Appl. Microbiol.** 24 (5): 393–396.

Hamsupo, K. 2005. **Production and formulation of lactic acid bacteria producing antimicrobial substances as chicken probiotic adjuncts.** Ph.D. Thesis, Kasetsart University.

_____, P. Sukyai, G. Loiseau, S. Nitisinprasert, D. Montet and P. Wanchaitanawong. 2005. Prediction on the stability of spray-dried *Lactobacillus reuteri* KUB-AC5 by arrhenius equation for long-term storage. **J. Microbiol. Biotechnol.** 15 (6): 1178–1182.

Copyright by Kasetsart University All rights reserved

Handley, P.S., D.W.S. Harty, J.E. Wyatt, C.R. Brown, J.P. Doran and A.C.C. Gibbs. 1987. A comparison of the adhesion, coaggregation and cell-surface hydrophobicity properties of fibrillar and fimbriate strains of *Streptococcus salivarius*. **J. Gen. Microbiol.** 133 (11): 3207–3217.

Havenaar, R., N.G. Brink and J.H.J. Huis In't Ved. 1992. Selection of strains for probiotics use. *In* Fuller, R., eds. **Probiotics: the scientific basis**. Chapman and Hall, London.

Heidebach, T., P. Först and U. Kulozik. 2010. Influence of casein-based microencapsulation on freeze-drying and storage of probiotic cells. **J. Food Eng.** 98 (3): 309–316.

Herreros, M.A., H. Sandoval, L. González, J.M. Castro, J.M. Fresno and M.E. Tornadijo. 2005. Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from armada cheese (a spanish goats' milk cheese). **Food Microbiol.** 22 (5): 455–459.

Hickson, M., A.L. D'Souza, N. Muthu, T.R. Rogers, S. Want and C. Rajkumar. 2007. Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. **BMJ.** 335 (7610): 80.

Higl, B., L. Kurtmann, C.U. Carlsen, J. Ratjen, P. Först, L.H. Skibsted, U. Kulozik and J. Risbo. 2007. Impact of water activity, temperature, and physical state on the storage stability of *Lactobacillus paracasei* ssp. *paracasei* freeze-dried in a lactose matrix. **Biotechnol. Prog.** 23 (4): 794–800.

Holzapfel, W.H. 2006. Introduction to prebiotics and probiotics, *In* I. Goktepe, V. K. Juneja and M. Ahmedna, eds. **Probiotics in food safety and human health.** Taylor & Francis, New York.

Copyright by Kasetsart University All rights reserved

Hou, J.-W., R.-C. Yu and C.-C. Chou. 2000. Changes in some components of soymilk during fermentation with bifidobacteria. **Food Res. Int.** 33 (5): 393–397.

Huang, Y. and M.C. Adams. 2004. *In vitro* assessment of the upper gastrointestinal tolerance of potential probiotic dairy propionibacteria. **Int. J. Food Microbiol.** 91 (3): 253–260.

Hwanhlem, N., S. Buradaleng, S. Wattanachant, S. Benjakul, A. Tani and S. Maneerat. 2011. Isolation and screening of lactic acid bacteria from thai traditional fermented fish (plasom) and production of plasom from selected strains. **Food Control** 22 (3–4): 401–407.

Iannitti, T. and B. Palmieri. 2010. Therapeutical use of probiotic formulations in clinical practice. **Clin. Nutr.** 29 (6): 701–725.

IDF Standard 026. 2004. **International Dairy Federation**. Dried milk-determination of moisture content (also ISO Standard 5537), Brussels, Belgium.

Ishibashi, N. and S. Shimamura. 1993. *Bifidobacteria*: research and development in japan. **Food Tech.** 47: 126–134.

_____, T. Tatematsu, S. Shimamura and M. Tomita. 1985. **Effect of water activity on the viability of freeze-dried *Bifidobacteria*.** International Institute of Refrigeration, Paris.

Jägerstad, M., J. Jastrebova and U. Svensson. 2004. Folates in fermented vegetables-a pilot study. **Labensm.-Wiss. U. Technol.** 37: 603–611.

Copyright by Kasetsart University All rights reserved

Kacem, M., H. Zadi-Karam and N. Karam. 2006. Detection and characterization of a novel bacteriocin produced by *Lactobacillus plantarum* OL9 against *Erwinia chrysanthemi* strains. **Nat. Appl. Sci. Ser. (Jordan)** 21: 159–171.

Karimi, R., A. Mortazavian and A. Da Cruz. 2011. Viability of probiotic microorganisms in cheese during production and storage: a review. **Dairy Sci. Technol.** 91 (3): 283–308.

Karmas, R., M. Pilar Buera and M. Karel. 1992. Effect of glass transition on rates of nonenzymic browning in food systems. **J. Agri. Food Chem.** 40 (5): 873–879.

Kaushik, J.K., A. Kumar, R.K. Duary, A.K. Mohanty, S. Grover and V.K. Batish. 2009. Functional and probiotic attributes of an indigenous isolate of *Lactobacillus plantarum*. **PLoS ONE** 4 (12): e8099.

Kawai, K., T. Hagiwara, R. Takai and T. Suzuki. 2005. The rate of non-enzymatic browning reaction in model freeze–dried food system in the glassy state. **Innov. Food Sci. Emerg. Technol.** 6 (3): 346–350.

Kearney, N., X.C. Meng, C. Stanton, J. Kelly, G.F. Fitzgerald and R.P. Ross. 2009. Development of a spray dried probiotic yoghurt containing *Lactobacillus paracasei* NFBC 338. **Int. Dairy J.** 19 (11): 684–689.

Khater, K.A.A., M.A. Ali and E.A.M. Ahmed. 2010. Effect of encapsulation on some probiotic criteria. **J. Am. Sci.** 6 (10): 836–845.

Kim, S.S. and S.R. Bhowmik. 1990. Survival of lactic acid bacteria during spray drying of plain yogurt. **J. Food Sci.** 55 (4): 1008–1010.

Copyright by Kasetsart University All rights reserved

Kim, W.S., L. Perl, J.H. Park, J.E. Tandianus and N.W. Duun. 2001. Assessment of stress response of the probiotic *Lactobacillus acidophilus*. **Curr. Microbiol.** 43: 346–350.

King, R.A., J.L. Broadbent and R.J. Head. 1996. Absorption and excretion of the soy isoflavone genistein in rats. **J. Nutr.** 126: 176–182.

King, V.A.-E., H.-J. Lin and C.-F. Liu. 1998. Accelerated storage testing of freeze-dried and controlled low-temperature vacuum dehydrated *Lactobacillus acidophilus*. **J. Gen. Appl. Microbiol.** 44: 161–165.

Kos, B., S. Jagoda, G. Jadranka and a.S. Matosic. 2000. Effect of protectors on viability of *Lactobacillus acidophilus* M92 in simulated gastrointestinal conditions. **Food Technol. Biotechnol.** 38 (2): 121–127.

_____., J. Šušković, S. Vuković, M. Šimpraga, J. Frece and S. Matošić. 2003. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. **J. Appl. Microbiol.** 94 (6): 981–987.

Kosin, B. and K.S. Rakshit. 2006. Microbial and processing criteria for production of probiotics: a review. **Food Technol. Biotechnol.** 44 (3): 371–379.

_____ and _____. 2010. Induction of heat tolerance in autochthonous and allochthonous thermotolerant probiotics for application to white shrimp feed. **Aquac.** 306 (1–4): 302–309.

Kourkoutas, Y., V. Xolias, M. Kallis, E. Bezirtzoglou and M. Kanellaki. 2005. *Lactobacillus casei* cell immobilization on fruit pieces for probiotic additive, fermented milk and lactic acid production. **Process Biochem.** 40 (1): 411–416.

Copyright by Kasetsart University All rights reserved

Krishnan, S., R. Bhosale and R.S. Singhal. 2005. Microencapsulation of cardamom oleoresin: evaluation of blends of gum arabic, maltodextrin and a modified starch as wall materials. **Carbohydr. Polym.** 61 (1): 95–102.

Krumnow, A.A., I.B. Sorokulova, E. Olsen, L. Globa, J.M. Barbaree and V.J. Vodyanoy. 2009. Preservation of bacteria in natural polymers. **J. Microbiol. Methods** 78 (2): 189–194.

Kurozawa, L.E., K.J. Park and M.D. Hubinger. 2009. Effect of maltodextrin and gum arabic on water sorption and glass transition temperature of spray dried chicken meat hydrolysate protein. **J. Food Eng.** 91 (2): 287–296.

Kurtmann, L., L.H. Skibsted and C.U. Carlsen. 2009a. Browning of freeze-dried probiotic bacteria cultures in relation to loss of viability during storage. **J. Agri. Food Chem.** 57 (15): 6736–6741.

_____, C.U. Carlsen, L.H. Skibsted and J. Risbo. 2009b. Water activity-temperature state diagrams of freeze-dried *Lactobacillus acidophilus* (La-5): Influence of physical state on bacterial survival during storage. **Biotechnol. Prog.** 25 (1): 265–270.

Labuza, T.P. and B. Fu. 1993. Growth kinetics for shelf-life prediction: theory and practice. **J. Ind. Microbiol. Biotechnol.** 12 (3): 309–323.

Labuza, T.P. and D. Riboh. 1982. Theory and application of arrhenius kinetics to the prediction of nutrient losses in foods. **Food Technol.** 36 (10): 66–74.

Lanciotti, R., F. Patrignani, F. Bagnolini, M.E. Guerzoni and F. Gardini. 2003. Evaluation of diacetyl antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. **Food Microbiol.** 20 (5): 537–543.

Copyright by Kasetsart University All rights reserved

Lapsiri, W. and P. Wanchaitanawong. 2012a. Protective effects of soybean, sesame and Job's Tears on the survival of fermented vegetable *Lactobacillus plantarum* under gastrointestinal tract conditions. **Afr. J. Microbiol. Res.** *In Press,* Accepted Manuscript.

_____., B. Bhandari and P. Wanchaitanawong. 2012b. Viability of *Lactobacillus plantarum* TISTR 2075 in different protectants during spray drying and storage. **Dry. Technol.** *In Press,* Accepted Manuscript.

_____., S. Nitisinprasert and P. Wanchaitanawong. 2011. *Lactobacillus plantarum* strains from fermented vegetables as potential probiotics. **Kasetsart J. (Nat. Sci.).** 45: 1071–1082.

Lee, Y.K. and S. Salminen. 2009. **Handbook of probiotics and prebiotics.** 2nd. John Wiley & Sons, Inc., New Jersey.

Leslie, S., E. Israeli, B. Lighthart, J. Crowe and L. Crowe. 1995. Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. **Appl. Environ. Microbiol.** 61 (10): 3592–3597.

Lian, W.-C., H.-C. Hsiao and C.-C. Chou. 2002. Survival of *Bifidobacteria* after spray-drying. **Int. J. Food Microbiol.** 74 (1–2): 79–86.

Liasi, S.A., T.I. Azmi, M.D. Hassan, M. Shuhaimi, M. Rosfarizan and A.B. Afriff. 2009. Antimicrobial activity and antibiotic sensitive of three isolated of lactic d bacteria from fermented fish product, Budu. **Malaysian J. Microbiol.** 5 (1): 33–37.

Lim, S.-M. 2009. Screening and characterization of probiotic lactic acid bacteria isolated from korean fermented foods. **J. Microbiol. Biotechnol.** 19 (2): 178–186.

Copyright by Kasetsart University All rights reserved

Lin, W.-H., C.-F. Hwang, L.-W. Chen and H.-Y. Tsen. 2006. Viable counts, characteristic evaluation for commercial lactic acid bacteria products. **Food Microbiol.** 23: 74–81.

Linders, L.J.M., W.F. Wolkers, F.A. Hoekstra and K. van 't Riet. 1997. Effect of added carbohydrates on membrane phase behavior and survival of dried *Lactobacillus plantarum*. **Cryobiol.** 35 (1): 31–40.

Liong, M.T. 2008. Safety of probiotics: translocation and infection. **Nutr. Rev.** 66 (6): 192–202.

Ljungh, A. and T. Wadstrom. 2006. Lactic acid bacteria as probiotic. **Curr. Issues Intestinal Microbiol.** 7: 73–90.

Lopez, H.W., A. Ouvry, E. Bervas, C. Guy, A. Messager, C. Demigne and C. Remesy. 2000. Strains of lactic acid bacteria isolated from sour doughs degrade phytic acid and improve calcium and magnesium solubility from whole wheat flour. **J. Agric. Food Chem.** 99: 179–186.

Lourens-Hattingh, A. and B.C. Viljoen. 2001. Yogurts as probiotic carrier food. **Int. Dairy. J.** 11: 1–17.

Madley, R. H. 2001. Probiotics, prebiotics & synbiotics: harnessing enormous potential. **Neutraceuticals World** (September): 50–77.

Mårtenson, O., R. Öste and O. Holst. 2002. The effect of yogurth culture on the survival of probiotic bacteria in oat-based, non-dairy products. **Food Res. Int.** 35: 775–784.

Mathur, S. and R. Singh. 2005. Antibiotic resistance in food lactic acid bacteria-a review. **Int. J. Food Microbiol.** 105 (3): 281–295.

Copyright by Kasetsart University All rights reserved

McDonald, L.C., H.P. Fleming and H.M. Hassan. 1990. Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. **Appl. Environ. Microbiol.** 56 (7): 2120–2124.

Meng, X.C., C. Stanton, G.F. Fitzgerald, C. Daly and R.P. Ross. 2008. Anhydrobiotics: the challenges of drying probiotic cultures. **Food Chem.** 106 (4): 1406–1416.

Mercenier, A., S. Pavan and B. Pot. 2003. Probiotics as biotherapeutic agents: present knowledge and future prospects. **Curr. Phar. Design** 8: 99–110.

Michel, C., T. P. Kravtchenko, A. David, S. Gueneau, F. Kozlowski and C. Cherbut. 1998. *In vitro* prebiotic effects of acacia gums onto the human intestinal microbiota depends on both botanical origin and environmental pH. **Anaerob.** 4(6): 257–266.

Michida, H., S. Tamalampudi, S.S. Pandiella, C. Webb, H. Fukuda and A. Kondo. 2006. Effect of cereal extracts and cereal fiber on viability of *Lactobacillus plantarum* under gastrointestinal tract conditions. **Biochem. Eng. J.** 28 (1): 73–78.

Mille, Y., J.-P. Obert, L. Beney and P. Gervais. 2004. New drying process for lactic bacteria based on their dehydration behavior in liquid medium. **Biotechnol. Bioeng.** 88 (1): 71–76.

Miller, G. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. **Anal. Chem.** 31: 426–428.

Copyright by Kasetsart University All rights reserved

Mizrahi, S. 2000. Accelerated shelf-life tests, pp. 107–125. *In* D. Kilcast and P. Subramaniam, eds. **The stability and shelf-life of food.** Woodhead Publishing Limited, Cambridge, England.

Molin, G. 2001. Probiotics in foods not containing milk or milk constituents, with special reference to *Lactobacillus plantarum* 299v. **Am. J. Clin. Nutr.** 73 (suppl) : 380S–385S.

Morelli, L. 2000. *In vitro* selection of probiotic *Lactobacilli*: a critcal appraisal. **Curr. Issues Intest. Microbiol.** 1 (2): 59–67.

Morgan, C.A., N. Herman, P.A. White and G. Vesey. 2006. Preservation of micro-organisms by drying: a review. **J. Microbiol. Methods** 66 (2): 183–193.

Mousavi, Z., S. Mousavi, S. Razavi, Z. Emam-Djomeh and H. Kiani. 2011. Fermentation of pomegranate juice by probiotic lactic acid bacteria. **World J. Microbiol. Biotechnol.** 27 (1): 123–128.

Muller, J.A., R.P. Ross, G.F. Fitzgerald and C. Stanton. 2009. Manufacture of probiotic bacteria, pp. 725–759. *In* D. Charalampopoulos and R. A. Rastall, eds. **Prebiotics and probiotics science and technology.** Springer, New York.

Nicolescu, C.L. and L.C. Buruleanu. 2010. Correlation of some substrate parameters in growing *Lactobacillus acidophilus* on vegetable and fruit cocktail juices. **Bulletin UASVM Agriculture** 67 (2): 352–359.

O'Callaghan, J. and S. Condon. 2000. Growth of lactococcus lactis strains at low water activity: Correlation with the ability to accumulate glycine betaine. **Int. J. Food Microbiol.** 55 (1–3): 127–131.

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O'Sullivan, D.J. 2006. Primary sources of probiotic cultures, pp. 91-107. *In* I. Goktepe, V. K. Juneja and M. Ahmed, eds. **Probiotics in food safety and human health.** CRC Taylor & Francis, New York.

Otero, M.C., M.C. Espeche and M.E. Nader-Macías. 2007. Optimization of the freeze-drying media and survival throughout storage of freeze-dried *Lactobacillus gasseri* and *Lactobacillus delbrueckii* subsp. *delbrueckii* for veterinarian probiotic applications. **Process Biochem.** 42 (10): 1406–1411.

Otieno, D.O., J.F. Ashton and N.P. Shah. 2007a. Isoflavone phytoestrogen degradation in fermented soymilk with selected β-glucosidase producing *L. acidophilus* strains during storage at different temperatures. **Int. J. Food Microbiol.** 115 (1): 79–88.

_____ and _____. 2007b. Role of microbial strain and storage temperatures in the degradation of isoflavone phytoestrogens in fermented soymilk with selected β-glucosidase producing *Lactobacillus casei* strains. **Food Res. Int.** 40 (3): 371–380.

Oyetayo, V.O. and F.L. Oyetayo. 2005. Potential of probiotics as biotherapeutic agents targeting the innate immune system. **Afr. J. Biotechnol.** 4 (2): 123–127.

Palzer, S. 2005. The effect of glass transition on the desired and undesired agglomeration of amorphous food powders. **Chem. Eng. Sci.** 60 (14): 3959–3968.

Patel, H.M., S.S. Pandiella, R.H. Wang and C. Webb. 2004a. Influence of malt, wheat, and barley extracts on the bile tolerance of selected strains of *Lactobacilli*. **Food Microbiol.** 21 (1): 83–89.

Copyright by Kasetsart University All rights reserved

Patel, H.M., R. Wang, O. Chandrashekar, S.S. Pandeilla and C. Webb. 2004b. Proliferation of *Lactobacillus plantarum* in solid-state fermentation of oats. **Biotechnol. Prog.** 20: 110–116.

Patist, A. and H. Zoerb. 2005. Preservation mechanisms of trehalose in food and biosystems. **Colloids and Surfaces B: Biointerfaces** 40 (2): 107–113.

Pehkonen, K.S., Y.H. Roos, S. Miao, R.P. Ross and C. Stanton. 2008. State transitions and physicochemical aspects of cryoprotection and stabilization in freeze-drying of *Lactobacillus rhamnosus* GG (LGG). **J. Appl. Microbiol.** 104 (6): 1732–1743.

Peighambardoust, S.H., A. Golshan Tafti and J. Hesari. 2011. Application of spray drying for preservation of lactic acid starter cultures: a review. **Trends Food Sci. Technol** 22 (2011): 215–224.

Pennacchia, C., D. Ercolini, G. Blaiotta, O. Pepe, G. Mauriello and F. Villani. 2004. Selection of *Lactobacillus* strains from fermented sausages for their potential use as probiotics. **Meat Sci.** 67: 309–317.

Picot, A. and C. Lacroix. 2004. Encapsulation of *Bifidobacteria* in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. **Int. Dairy J.** 14 (6): 505–515.

Potts, M. 1994. Desiccation tolerance of prokaryotes. **Microbiol. Rev.** 58 (4): 755–805.

_____. 2001. Desiccation tolerance: a simple process? **Trends Microbiol.** 9 (11): 553–559.

Copyright by Kasetsart University All rights reserved

Prado, F.C., J.L. Parada, A. Pandey and C.R. Soccol. 2008. Trends in non-dairy probiotic beverages. **Food Res. Int.** 41 (2): 111–123.

Prasad, J., P. McJarrow and P. Gopal. 2003. Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. **Appl. Environ. Microbiol.** 69 (2): 917–925.

Pyo, Y.-H., T.-C. Lee and Y.-C. Lee. 2005. Enrichment of bioactive isoflavones in soymilk fermented with β-glucosidase-producing lactic acid bacteria. **Food Res. Int.** 38 (5): 551–559.

Rahman, M., W.-S. Kim, H. Kumura and K.-i. Shimazaki. 2008. Autoaggregation and surface hydrophobicity of *Bifidobacteria*. **World J. Microbiol. Biotechnol.** 24 (8): 1593–1598.

Rakin, M., M. Vukasinovic, S. Siler-Marinkovic and M. Maksimovic. 2007. Contribution of lactic acid fermentation to improved nutritive quality vegetable juices enriched with brewer's yeast autolysate. **Food Chem.** 100 (2): 599–602.

Ranadheera, R.D.C.S., S.K. Baines and M.C. Adams. 2010. Importance of food in probiotic efficacy. **Food Res. Int.** 43 (1): 1–7.

Rautava, S., M. Kalliomäki and E. Isolauri. 2002. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. **J. Allergy Clin. Immunol.** 109: 119–121.

Reddy, K.B.P.K., A.N. Madhu and S.G. Prapulla. 2009. Comparative survival and evaluation of functional probiotic properties of spray-dried lactic acid bacteria. **Int. J. Dairy Technol.** 62 (2): 240–248.

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Rivera-Espinoza, Y. and Y. Gallardo-Navarro. 2010. Non-dairy probiotic products. **Food Microbiol.** 27 (1): 1–11.

Riveros, B., J. Ferrer and R. Bórquez. 2009. Spray drying of a vaginal probiotic strain of *Lactobacillus acidophilus*. **Dry Technol.** 27 (1): 123–132.

Rodríguez, H., J.A. Curiel, J.M. Landete, B. de las Rivas, F.L. de Felipe, C. Gómez-Cordovés, J.M. Mancheño and R. Muñoz. 2009. Food phenolics and lactic acid bacteria. **Int. J. Food Microbiol.** 132 (2–3): 79–90.

Roos, Y.H. 2001. Water acitivity and plasticization, pp. 3-35. *In* N. A. Michael Eskin and D. S. Robinson, eds. **Food shelf life stability: Chemical, biochemical and microbiological changes.** CRC Press, London.

Roos, Y. 1995. **Phase transitions in foods.** Academic Press, INC, California. 360 p.

_____. 2006. Water activity and glass transition, pp. 29-45. *In*, eds. **Water properties of food, pharmaceutical, and biological materials.** CRC Press Taylor & Francis Group, New York.

_____, K. Jouppila and B. Zielasko. 1996. Non-enzymatic browning-induced water plasticization. **J. Therm. Anal. Calorim.** 47 (5): 1437–1450.

Saarela, M., Gunnar Mogensen, Rangne Fondén, Jaana Mättö and T. Mattila-Sandholm. 2000. Probiotic bacteria: safety, functional and technological properties. **J. Biotechnol.** 84: 197–215.

Sablani, S.S., L. Bruno, S. Kasapis and R.M. Symaladevi. 2009. Thermal transitions of rice: development of a state diagram. **J. Food Eng.** 90 (1): 110–118.

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Salminen, S., A. von Wright, L. Morelli, P. Marteau, D. Brassart, W.M. de Vos, R. Fondén, M. Saxelin, K. Collins, G. Mogensen, S.-E. Birkeland and T. Mattila-Sandholm. 1998. Demonstration of safety of probiotics - a review. **Int. J. Food Microbiol.** 44 (1–2): 93–106.

Sanders, J.W., G. Venema and J. Kok. 1999. Environmental stress responses in *Lactococcus lactis*. **FEMS Microbiol. Rev.** 23 (4): 483–501.

Sanders, M.E. 2003. Probiotics: considerations for human health. **Nutr. Rev.** 61 (3): 91–99.

Santivarangkna, C., B. Higl and P. Foerst. 2008a. Protection mechanisms of sugars during different stages of preparation process of dried lactic acid starter cultures. **Food Microbiol.** 25 (3): 429–441.

_____, M. Aschenbrenner, U. Kulozik and P. Foerst. 2011. Role of glassy state on stabilities of freeze-dried probiotics. **J. Food Sci.** 76 (8): R152–R156.

_____, U. Kulozik and P. Foerst. 2007. Alternative drying processes for the industrial preservation of lactic acid starter cultures. **Biotechnol. Prog.** 23 (2): 302–315.

_____, U. Kulozik and P. Foerst. 2008b. Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. **J. Appl. Microbiol.** 105: 1–13.

Saxelin, M., S. Tynkkynen, T. Mattila-Sandholm and W.M. de Vos. 2005. Probiotic and other functional microbes: from markets to mechanisms. **Curr. Opin. Biotechnol.** 16 (2): 204–211.

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Schoug, Å., D. Mahlin, M. Jonson and S. Håkansson. 2010. Differential effects of polymers PVP 90 and Ficoll 400 on storage stability and viability of *Lactobacillus coryniformis* SI3 freeze-dried in sucrose. **J. Appl. Microbiol.** 108 (3): 1032–1040.

Selmer-Olsen, E., S.E. Birkeland and T. Sørhaug. 1999. Effect of protective solutes on leakage from and survival of immobilized *Lactobacillus* subjected to drying, storage and rehydration. **J. Appl. Microbiol.** 87 (3): 429–437.

Senthuran, A., V. Santhuran, H.K. Rajni and B. Mattiasson. 1999. Lactic acid production by immobilized *Lactobacillus casei* in recycle batch reactor: a step towards optimization. **J. Biotechnol.** 73: 61–70.

Serrazanetti, D.I., M.E. Guerzoni, A. Corsetti and R. Vogel. 2009. Metabolic impact and potential exploitation of the stress reactions in *Lactobacilli*. **Food Microbiol.** 26 (7): 700–711.

Sheehan, V.M., P. Ross and G.F. Fitzgerald. 2007. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. **Innov. Food Sci. Emerg. Technol.** 8: 279–284.

_____, R.D. Sleator, G.F. Fitzgerald and C. Hill. 2006. Heterologous expression of betl, a betaine uptake system, enhances the stress tolerance of *Lactobacillus salivarius* UCC 118. **Appl. Environ. Microbiol.** 72 (3): 2170–2177.

Shirai, K., I. Guerrero, S. Huerta, G. Saucedo, A. Castillo, R.O. Gonzalez and G.M. Hall. 2001. Effect of initial glucose concentration and inoculation level of lactic acid bacteria in shrimp waste ensilation. **Enzym. Microb. Technol.** 28 (28): 446–452.

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Shrestha, A.K., T. Howes, B.P. Adhikari and B.R. Bhandari. 2007a. Water sorption and glass transition properties of spray dried lactose hydrolysed skim milk powder. **LWT - Food Sci. Technol.** 40 (9): 1593–1600.

_____, T. Ua-arak, B.P. Adhikari, T. Howes and B.R. Bhandari. 2007b. Glass transition behavior of spray dried orange juice powder measured by differential scanning calorimetry (DSC) and thermal mechanical compression test (TMCT). **Int. J. Food Prop.** 10 (3): 661–673.

Silva, J., A.S. Carvalho, V. Ferreira, R. Vitorino, F. Amado, P. Domingues, P. Teixeira and P.A. Gibbs. 2005. Effect of the pH of growth on the survival of *Lactobacillus delbrueckii* subsp. *bulgaricus* to stress conditions during spray-drying. **J. Appl. Microbiol.** 98: 775–782.

_____, R. Freixo, P. Gibbs and P. Teixeira. 2011. Spray-drying for the production of dried cultures. **Int. J. Dairy Technol.** 64 (3): 321–335.

Simakachorn, N., V. Pichaipat, P. Rithipornpaisarn, C. Kongkaew, P. Tongpradit and W. Varavithya. 2000. Clinical evaluation of the addition of lyophilized, heat-killed *Lactobacillus acidophilus* LB to oral rehydration therapy in the treatment of acute diarrhea in children **J. Ped. Gastroenterol. Nutr.** 30: 68–72.

Simpson, P.J., C. Stanton, G.F. Fitzgerald and R.P. Ross. 2005. Intrinsic tolerance of *Bifidobacterium* species to heat and oxygen and survival following spray drying and storage. **J. Appl. Microbiol.** 99 (3): 493–501.

Soccol, C.R., L.P. de Souza Vandenberghe, M.R. Spier, A.B.P. Medeiros, C.T. Yamaguishi, J. De Dea Lindner, A. Pan and V. Thomaz-Soccol. 2010. The potential of probiotics: a review. **Food Technol.** 48 (4): 413–434.

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Sorokulova, I.B., A.A. Krumnow, S. Pathirana, A.J. Mandell and V. Vodyanoy. 2008. Novel methods for storage stability and release of *Bacillus* spores. **Biotechnol. Prog.** 24: 1147–1153.

Strasser, S., M. Neureiter, M. Geppl, R. Braun and H. Danner. 2009. Influence of lyophilization, fluidized bed drying, addition of protectants, and storage on the viability of lactic acid bacteria. **J. Appl. Microbiol.** 107 (1): 167–177.

Succi, M., P. Tremonte, A. Reale, E. Sorrentino, L. Grazia, S. Pacifico and R. Coppola. 2005. Bile salt and acid tolerance of *Lactobacillus rhamnosus* strains isolated from parmigiano reggiano cheese. **FEMS Microbiol. Lett.** 244 (1): 129–137.

Sung-Mee, L. and I. Dong-Soon. 2009. Screening and characterization of probiotic lactic acid bacteria isolated from korean fermented foods. **J. Microbiol. Biotechnol.** 19 (2): 178–186.

Sunny-Roberts, E.O. and D. Knorr. 2009. The protective effect of monosodium glutamate on survival of *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* E-97800 (E800) strains during spray-drying and storage in trehalose-containing powders. **Int. Dairy J.** 19 (4): 209–214.

_____ and _____. 2011. Cellular injuries on spray-dried *Lactobacillus rhamnosus* GG and its stability during food storage. **Nutr. Food Sci.** 41 (3): 191–200.

Taheri, H.R., H. Moravej, F. Tabandeh, M. Zaghari and M. Shivazad. 2009a. Screening of lactic acid bacteria toward their selection as a source of chicken probiotic. **Poultr. Sci.** 88: 1586–1593.

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Taheri, H.R., F. Tabandeh, H. Moravej, M. Zaghari, M. Shivazad and P. Shariati. 2009b. Potential probiotic of *Lactobacillus johnsonii* LT171 for chicken nutrition. **Afr. J. Biotechnol.** 8 (21): 5833–5837.

Takeda, K. and K. Okumura. 2007. Effects of a fermented milk drink containing *Lactobacillus casei* strain shirota on the human NK-cell activity. **J. Nutr.** 137 (3): 791S–793S.

Tang, A.L., N.P. Shan, G. Wilcox, K.Z. Walker and L. Stojanovska. 2007. Fermentation of calcium-fortified soymilk with *Lactobacillus*: effects on calcium solubility, isoflavone conversion, and production of organic acids. **J. Food Sci.** 72 (Nr. 9): M431–M436.

Teixeira, J.A., H. Castro, F.X. Malcata and R.M. Kirby. 1995a. Survival of *Lactobacillus delbrueckii* ssp. *bulgaricus* following spray-drying. **J. Dairy Sci.** 78: 1025–1031.

Teixeira, P., H. Castro and R. Kirby. 1995b. Spray drying as a method for preparing concentrated cultures of *Lactobacillus bulgaricus*. **J. Appl. Bacteriol.** 78: 456–462.

_____ and _____. 1996. Evidence of membrane lipid oxidation of spray-dried *Lactobacillus bulgaricus* during storage. **Lett. Appl. Microbiol.** 22 (1): 34–38.

_____, C. Mohácsi-Farkas and R. Kirby. 1997. Identification of sites of injury in *Lactobacillus bulgaricus* during heat stress. **J. Appl. Micro.** 83 219–226.

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Timbuntam, W., K. Sriroth and Y. Tokiwa. 2006. Lactic acid production from sugar-cane juice by a newly isolated *Lactobacillus* sp. **Biotechnol. Lett.** 28 (11): 811–814.

Toda, T., A. Sakamoto, T. Takayanagi and K. yokotsuka. 2001. Changes in isoflavone compositions of soybeans during the soaking process. **Food Sci. Technol. Res.** 6: 314–319.

Toledo, N., J. Ferrer and R. Bórquez. 2010. Drying and storage stability of a probiotic strain incorporated into a fish feed formulation. **Dry. Technol.** 28: 508–516.

Tonon, R.V., A.F. Baroni, C. Brabet, O. Gibert, D. Pallet and M.D. Hubinger. 2009. Water sorption and glass transition temperature of spray dried açai (*Euterpe oleracea* mart.) juice. **J. Food Eng.** 94 (3–4): 215–221.

Truong Thuc, T., S. Fukai, V. Truong and B. Bhandari. 2010. Measurement of glass-rubber transition temperature of rice by thermal mechanical compression test (TMCT). **Int. J. Food Prop.** 13: 176–183.

Truong, V., B.R. Bhandari and T. Howes. 2005. Optimization of cocurrent spray drying process for sugar-rich foods. Part II-optimization of spray drying process based on glass transition concept. **J. Food Eng.** 71 (1): 66–72.

Tsai, C.C., L.F. Huang, C.C. Lin and H.Y. Tsen. 2004. Antagonistic activity against *Helicobacter pylori* infection *in vitro* by a strain of *Enterococcus faecium* TM39. **Int. J. Food Microbiol.** 96: 1–12.

Tsen, H.Y., Y.P. Lin and V. An-Erl King. 2003. Banana puree fermentation by *Lactobacillus acidophilus* immobilized in Ca-alginate. **J. Gen. Appl. Microbiol.** 49: 357–361.

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Tsen, J.-H., Y.-P. Lin, H.-Y. Huang and V.A.-E. King. 2007. Accelerated storage testing of freeze-dried immobilized *Lactobacillus acidophilus*-fermented banana media. **J. Food Process. Preserv.** 31 (6): 688–701.

Tuomola, E., R. Crittenden, M. Playne, E. Isolauri and S. Salminen. 2001. Quality assurance criteria for probiotic bacteria. **Am. J. Clin. Nutr** 73 (suppl): 393S–398S.

Valerio, F., P. De Bellis, S.L. Lonigro, L. Morelli, A. Visconti and P. Lavermicocca. 2006. *In vitro* and *in vivo* survival and transit tolerance of potentially probiotic strains carried by artichokes in the gastrointestinal tract. **Appl. Environ. Microbiol.** 72 (4): 3042–3045.

Vasiljevic, T. and N.P. Shah. 2008. Probiotics-from metchnikoff to bioactives. **Int. Dairy J.** 18 (7): 714–728.

van de Guchte, M., P. Serror, C. Chervaux, T. Smokvina, S. Ehrlich and E. Maguin. 2002. Stress responses in lactic acid bacteria. **Antonie van Leeuwenhoek** 82 (1): 187–216.

Vlková, E., V. Rada, M. Šmehilová and J. Killer. 2008. Auto-aggregation and co-aggregation ability in *Bifidobacteria* and *Clostridia*. **Folia Microbiol.** 53 (3): 263–269.

_____, P. Popelárová, I. Trojanová and J. Killer. 2006. Antimicrobial susceptibility of *Bifidobacteria i*solated from gastrointestinal tract of calves. **Livest. Sci.** 105 (1–3): 253–259.

Wang, C.-Y., S.-C. Wu, C.-C. Ng and Y.-T. Shyu. 2010. Effect of lactobacillus-fermented adlay-based milk on lipid metabolism of hamsters fed cholesterol-enriched diet. **Food Res. Int.** 43 (3): 819–824.

Copyright by Kasetsart University All rights reserved

Wang, C.-Y., C.-C. Ng, H. Su, W.-S. Tzeng and Y.-T. Shyu. 2009. Probiotic potential of noni juice fermented with lactic acid bacteria and *Bifidobacteria.* **Int. J. Food Sci. Nutr.** 60 (S6): 98–106.

Wang, N.-F., Y.-H. Shi, J. Sun and G.-W. Le. 2007. Evaluation of peanut flour fermented with lactic acid bacteria as a probiotic food. **Food Sci. Technol Int.** 13 (6): 469–475.

Wang, Y.-C., R.-C. Yu and C.-C. Chou. 2002. Growth and survival of *Bifidobacteria* and lactic acid bacteria during the fermentation and storage of cultured soymilk drinks. **Food Microbiol.** 19 (5): 501–508.

_____. 2004. Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. **Int. J. Food Microbiol.** 93 (2): 209–217.

Wei, Q.-K., T.-R. Chen and J.-T. Chen. 2007. Using of *Lactobacillus* and *Bifidobacterium* to product the isoflavone aglycones in fermented soymilk. **Int. J. Food Microbiol.** 117 (1): 120–124.

Wirunpan, M. 2011. **Survival of *Lactobacillus lactis* 1464 in shrimp feed pellet during drying process and storage.** M.Sc. Thesis, Kasetsart University.

Yadav, A.K., A.B. Chaudhari and R.M. Kothari. 2009. Enhanced viability of *Bacillus coagulans* after spray drying with calcium lactate, storage and re-hydration. **Indian J. Chem. Technol.** 16 (November): 519–522.

Yeo, S.-K. and M.-T. Liong. 2010. Effect of prebiotics on viability and growth characteristics of probiotics in soymilk. **J. Sci. Food Agri.** 90 (2): 267–275.

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Ying, D.Y., M.C. Phoon, L. Sanguansri, R. Weerakkody, I. Burgar and M.A. Augustin. 2010. Microencapsulated *Lactobacillus rhamnosus* GG powders: relationship of powder physical properties to probiotic survival during storage. **J. Food Sci.** 75 (9): E588–E595.

Yoon, K.Y., E.E. Woodams and Y.D. Hang. 2004. Probiotication of tomato juice by lactic acid bacteria. **J. Microbiol.** 42: 315-–318.

_____ and _____. 2005. Fermentation of beet juice by beneficial lactic acid bacteria. **Lebensmittel-Wissenschaft und-Technologie** 38 (1): 73–75.

_____and_____. 2006. Production of probiotic cabbage juice by lactic acid bacteria. **Bioresour. Technol.** 97: 1427–1430.

Zayed, G. and Y.H. Roos. 2004. Influence of trehalose and moisture content on survival of *Lactobacillus salivarius* subjected to freeze-drying and storage. **Process Biochem.** 39 (9): 1081–1086.

Zhao, G. and G. Zhang. 2005. Effect of protective agents, freezing temperature, rehydration media on viability of malolactic bacteria subjected to freeze-drying. **J. Appl. Microbiol.** 99 (2): 333–338.

Zhou, J.S., C.J. Pillidge, P.K. Gopal and H.S. Gill. 2005. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. **Int. J. Food Microbiol.** 98 (2): 211–217.

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APPENDICES

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Appendix A

Preparation of fermented vegetables

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Preparation of fermented vegetables

The vegetables including Chinese cabbage, beetroot, pumpkin, carrot and spring onion were selected for fermentation. All vegetables were chopped, shredded or sliced into small pieces and mixed with 2.5% (w/w) salt, 2.5% (w/w) table sugar and 2.5% (w/w) water from washing rice. Then, the mixed vegetables were placed in a glass jar and poured with hot water until full. A chopstick or knife was used to poke down into the jar (around the sides) to remove air bubbles. Finally, the glass lid was close over the jar. The fermentation was carried out at 37 °C for 7 days.

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Appendix B

Lactic acid production, fermentation type and growth under different pH, NaCl and temperatures of 82 isolates

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Appendix Table B1 Lactic acid production, fermentation type and growth under different pH, NaCl and temperatures of isolated strains.

No.	Code No.	Lactic acid production (% ± S.D.)	Fermentative Type	Growth of test strains in different conditions													
				NaCl						pH					Temperature		
				0%	2%	4%	6%	8%	10%	2	4	6	8	10	20°C	37°C	45°C
1	**PKWA6-1**	**2.24 ± 0.00**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
2	PKWA6-2	2.21 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
3	PKWA6-3	2.21 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
4	PKWA6-4	1.99 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
5	PKWA6-5	2.02 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
6	PKWA6-6	1.99 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
7	PKWA6-7	2.07 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
8	PKWA6-8	1.97 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
9	PKWA6-9	1.97 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
10	PKWA6-10	2.04 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
11	PKWA6-11	2.02 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
12	PKWA6-12	2.02 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
13	PKWA6-13	1.92 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w

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Appendix Table B1 (Continued)

No.	Code No.	Lactic acid production (% ± S.D.)	Fermentative Type	Growth of test strains in different conditions													
				NaCl						pH					Temperature		
				0%	2%	4%	6%	8%	10%	2	4	6	8	10	20°C	37°C	45°C
14	PKWA6-14	1.99 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
15	PKWA6-15	2.02 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
16	PKWA6-16	2.02 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
17	**PKWA7-1**	**2.22 ± 0.02**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
18	PKWA7-2	1.94 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
19	PKWA7-3	2.00 ± 0.02	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
20	PKWA7-4	2.13 ± 0.05	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
21	PKWA7-5	2.09 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
22	PKWA7-6	2.04 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
23	PKWA7-7	2.16 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
24	PKWA7-8	2.19 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
25	PKWA7-9	2.16 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
26	PKWA7-10	2.02 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w

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Appendix Table B1 (Continued)

No.	Code No.	Lactic acid production (% ± S.D.)	Fermentative Type	Growth of test strains in different conditions													
				NaCl						pH					Temperature		
				0%	2%	4%	6%	8%	10%	2	4	6	8	10	20°C	37°C	45°C
27	PKWA7-11	1.99 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
28	PKWA7-12	2.11 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
29	**PKWB6-3**	**2.20 ± 0.02**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
30	PKWB6-4	2.16 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
31	PKWB6-5	2.04 ± 0.21	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
32	PKWB6-6	2.19 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
33	PKWB6-7	2.16 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
34	PKWB6-8	2.11 ± 0.10	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
35	PKWB6-9	2.07 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
36	PKWB6-10	2.16 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
37	PKWB6-11	2.07 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
38	**PKWB6-12**	**2.21 ± 0.03**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
39	PKWB6-13	2.14 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w

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Appendix Table B1 (Continued)

No.	Code No.	Lactic acid production (% ± S.D.)	Fermentative Type	Growth of test strains in different conditions													
				NaCl						pH					Temperature		
				0%	2%	4%	6%	8%	10%	2	4	6	8	10	20°C	37°C	45°C
40	PKWB6-14	2.04 ± 0.10	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
41	PKWB6-15	2.09 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
42	PKWB6-16	2.09 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
43	PKWB6-17	2.19 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
44	PKWB6-18	2.11 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
45	**PKWB7-1**	**2.22 ± 0.02**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
46	**PKWB7-2**	**2.24 ± 0.03**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
47	**PKWC6-1**	**2.24 ± 0.00**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
48	PKWC6-2	2.16 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
49	PKWC6-3	2.19 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
50	PKWC6-4	2.19 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
51	PKWC6-5	2.19 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
52	PKWC6-6	2.09 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w

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Appendix Table B1 (Continued)

No.	Code No.	Lactic acid production (% ± S.D.)	Fermentative Type	Growth of test strains in different conditions													
				NaCl						pH					Temperature		
				0%	2%	4%	6%	8%	10%	2	4	6	8	10	20°C	37°C	45°C
53	PKWC6-7	1.94 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
54	PKWC6-8	2.14 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
55	**PKWC6-9**	**2.22 ± 0.00**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
56	PKWC6-10	2.16 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
57	PKWC6-11	2.14 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
58	PKWC6-12	2.14 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
59	PKW**C7-1**	**2.24 ± 0.00**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
60	PKWC7-2	2.14 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
61	PKWC7-3	2.16 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
62	PKWC7-4	2.16 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
63	PKWC7-5	2.14 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
64	PKWC7-6	2.14 ± 0.05	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
65	PKWC7-7	2.14 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-

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Appendix Table B1 (Continued)

No.	Code No.	Lactic acid production (% ± S.D.)	Fermentative Type	Growth of test strains in different conditions													
				NaCl						pH					Temperature		
				0%	2%	4%	6%	8%	10%	2	4	6	8	10	20°C	37°C	45°C
66	PKW**C8-1**	**2.22 ± 0.07**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
67	PKWD6-1	2.14 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
68	PKWD6-2	2.09 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
69	PKWD6-3	2.09 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
70	PKWD6-4	2.09 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
71	PKWD6-5	2.09 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
72	PKWD6-6	2.13 ± 0.05	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
73	PKWD6-7	2.11 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
74	PKWD6-8	2.09 ± 0.07	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
75	PKWD6-9	2.11 ± 0.03	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-
76	**PKWD6-10**	**2.31 ± 0.00**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
77	**PKWD7-1**	**2.26 ± 0.00**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
78	**PKWD7-2**	**2.33 ± 0.00**	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w

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Appendix Table B1 (Continued)

No.	Code No.	Lactic acid production (% ± S.D.)	Fermentative Type	Growth of test strains in different conditions													
				NaCl						pH					Temperature		
				0%	2%	4%	6%	8%	10%	2	4	6	8	10	20°C	37°C	45°C
79	PKWD7-3	2.14 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
80	PKWD7-4	2.14 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
81	PKWD7-5	2.19 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	w
82	PKWD7-6	2.19 ± 0.00	Homo	+	+	+	+	w	-	-	+	+	+	+	w	+	-

+: good growth; w: fair growth; - : no growth

Homo: Homofermentative

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Appendix C

Biochemical test of microorganisms isolated from fermented vegetable

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Appendix Table C1 Biochemical test of *L. plantarum* PKWA6-1[*] or TISTR 2070[**].

Test	Results	Test	Results	
Aerobe	+	PY base-Dextrose	3.86	+
Anaerobe	+	PY base-Fructose	3.93	+
Hemolysis zone	Beta	PY base-Lactose	4.09	+
Gram stain	Positive rod	PY base-Maltose	3.88	+
Spore	Non spore	PY base-Mannose	3.95	+
Catalase	-	PY base-Mannitol	4.84	+
Oxidase	-	PY base-Salicin	4.10	+
H_2S	-	PY base-Sucrose	4.11	+
Indole	-	PY base-Xylose	5.90	W/+
Motile	-	PY base-Arabinose	4.00	+
Litmus milk	Clot	PY base-Starch	4.44	+
Nitrate	+	Starch hydrolysis	-	
Gelatine	-	Lipase	-	
Urease	-	Lecithinase	-	
Esculin hydrolysis	+	MRS agar	Small opaque white colony	

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C2 Biochemical test of *L. plantarum* PKWA7-1[*] or TISTR 2071[**].

Test	Result	Test	Result
Aerobe	+	PY base-Dextrose	3.95 +
Anaerobe	+	PY base-Fructose	3.96 +
Hemolysis zone	Beta	PY base-Lactose	4.19 +
Gram stain	Positive rod	PY base-Maltose	3.89 +
Spore	Non spore	PY base-Mannose	4.07 +
Catalase	-	PY base-Mannitol	4.88 +
Oxidase	-	PY base-Salicin	4.02 +
H_2S	-	PY base-Sucrose	4.23 +
Indole	-	PY base-Xylose	5.94 W/+
Motile	-	PY base-Arabinose	4.03 +
Litmus milk	Clot	PY base-Starch	4.54 +
Nitrate	+	Starch hydrolysis	-
Gelatine	-	Lipase	-
Urease	-	Lecithinase	-
Esculin hydrolysis	+	MRS agar	Small opaque white colony

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C3 Biochemical test of *L. plantarum* PKWB6-3[*] or TISTR 2072[**].

Test	Result	Test	Result
Aerobe	+	PY base-Dextrose	3.98 +
Anaerobe	+	PY base-Fructose	4.05 +
Hemolysis zone	Beta	PY base-Lactose	4.64 +
Gram stain	Positive rod	PY base-Maltose	3.98 +
Spore	Non spore	PY base-Mannose	3.87 +
Catalase	-	PY base-Mannitol	4.82 +
Oxidase	-	PY base-Salicin	4.08 +
H_2S	-	PY base-Sucrose	3.89 +
Indole	-	PY base-Xylose	5.74 W/+
Motile	-	PY base-Arabinose	4.58 +
Litmus milk	Clot	PY base-Starch	4.48 +
Nitrate	+	Starch hydrolysis	-
Gelatine	-	Lipase	-
Urease	-	Lecithinase	-
Esculin hydrolysis	+	MRS agar	Small opaque white colony

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C4 Biochemical test of *L. plantarum* PKWB6-12[*] or TISTR 2073[**].

Test	Result	Test	Result	
Aerobe	+	PY base-Dextrose	3.88	+
Anaerobe	+	PY base-Fructose	3.88	+
Hemolysis zone	Beta	PY base-Lactose	4.73	+
Gram stain	Positive rod	PY base-Maltose	4.07	+
Spore	Non spore	PY base-Mannose	3.87	+
Catalase	-	PY base-Mannitol	4.82	+
Oxidase	-	PY base-Salicin	4.17	+
H_2S	-	PY base-Sucrose	4.54	+
Indole	-	PY base-Xylose	5.85	W/+
Motile	-	PY base-Arabinose	4.75	+
Litmus milk	Clot	PY base-Starch	4.54	+
Nitrate	+	Starch hydrolysis	-	
Gelatine	-	Lipase	-	
Urease	-	Lecithinase	-	
Esculin hydrolysis	+	MRS agar	Small opaque white colony	

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C5 Biochemical test of *L. plantarum* PKWB7-1* or TISTR 2074**.

Test	Result	Test	Result
Aerobe	+	PY base-Dextrose	3.90 +
Anaerobe	+	PY base-Fructose	4.04 +
Hemolysis zone	Beta	PY base-Lactose	4.89 +
Gram stain	Positive rod	PY base-Maltose	4.00 +
Spore	Non spore	PY base-Mannose	3.87 +
Catalase	-	PY base-Mannitol	4.52 +
Oxidase	-	PY base-Salicin	4.09 +
H_2S	-	PY base-Sucrose	3.90 +
Indole	-	PY base-Xylose	5.80 W/+
Motile	-	PY base-Arabinose	4.87 +
Litmus milk	Clot	PY base-Starch	4.65 +
Nitrate	+	Starch hydrolysis	-
Gelatine	-	Lipase	-
Urease	-	Lecithinase	-
Esculin hydrolysis	+	MRS agar	Small opaque white colony

*: Former identified code number

**: TISTR number obtained from MIRCEN

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Appendix Table C6 Biochemical test of *L. plantarum* PKWB7-2[*] or TISTR 2075 [**].

Test	Result	Test	Result
Aerobe	+	PY base-Dextrose	3.91 +
Anaerobe	+	PY base-Fructose	4.12 +
Hemolysis zone	Beta	PY base-Lactose	4.14 +
Gram stain	Positive rod	PY base-Maltose	5.21 +
Spore	Non spore	PY base-Mannose	3.98 +
Catalase	-	PY base-Mannitol	4.64 +
Oxidase	-	PY base-Salicin	4.09 +
H_2S	-	PY base-Sucrose	4.07 +
Indole	-	PY base-Xylose	5.91 W/+
Motile	-	PY base-Arabinose	4.30 +
Litmus milk	Clot	PY base-Starch	4.63 +
Nitrate	+	Starch hydrolysis	-
Gelatine	-	Lipase	-
Urease	-	Lecithinase	-
Esculin hydrolysis	+	MRS agar	Small opaque white colony

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C7 Biochemical test of *L. plantarum* PKWC6-1[*] or TISTR 2076 [**].

Test	Result	Test	Result
Aerobe	+	PY base-Dextrose	4.28 +
Anaerobe	+	PY base-Fructose	4.16 +
Hemolysis zone	Beta	PY base-Lactose	4.34 +
Gram stain	Positive rod	PY base-Maltose	4.07 +
Spore	Non spore	PY base-Mannose	4.39 +
Catalase	-	PY base-Mannitol	4.81 +
Oxidase	-	PY base-Salicin	4.07 +
H_2S	-	PY base-Sucrose	4.37 +
Indole	-	PY base-Xylose	5.97 W/+
Motile	-	PY base-Arabinose	4.58 +
Litmus milk	Clot	PY base-Starch	4.66 +
Nitrate	+	Starch hydrolysis	-
Gelatine	-	Lipase	-
Urease	-	Lecithinase	-
Esculin hydrolysis	+	MRS agar	Small opaque white colony

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C8 Biochemical test of *L. plantarum* PKWC6-9* or TISTR 2077**.

Test	Result	Test	Result	
Aerobe	+	PY base-Dextrose	4.24	+
Anaerobe	+	PY base-Fructose	4.05	+
Hemolysis zone	Beta	PY base-Lactose	4.42	+
Gram stain	Positive rod	PY base-Maltose	3.96	+
Spore	Non spore	PY base-Mannose	4.21	+
Catalase	-	PY base-Mannitol	4.82	+
Oxidase	-	PY base-Salicin	4.23	+
H_2S	-	PY base-Sucrose	4.37	+
Indole	-	PY base-Xylose	5.91	W/+
Motile	-	PY base-Arabinose	4.44	+
Litmus milk	Clot	PY base-Starch	5.18	+
Nitrate	+	Starch hydrolysis	-	
Gelatine	-	Lipase	-	
Urease	-	Lecithinase	-	
Esculin hydrolysis	+	MRS agar	Small opaque white colony	

*: Former identified code number

**: TISTR number obtained from MIRCEN

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Appendix Table C9 Biochemical test of *L. plantarum* PKWC7-1[*] or TISTR 2078[**].

Test	Result	Test	Result
Aerobe	+	PY base-Dextrose 4.28	+
Anaerobe	+	PY base-Fructose 4.16	+
Hemolysis zone	Beta	PY base-Lactose 4.34	+
Gram stain	Positive rod	PY base-Maltose 4.07	+
Spore	Non spore	PY base-Mannose 4.39	+
Catalase	-	PY base-Mannitol 4.81	+
Oxidase	-	PY base-Salicin 4.07	+
H_2S	-	PY base-Sucrose 4.37	+
Indole	-	PY base-Xylose 5.97	W/+
Motile	-	PY base-Arabinose 4.58	+
Litmus milk	Clot	PY base-Starch 4.66	+
Nitrate	-	Starch hydrolysis	-
Gelatine	-	Lipase	-
Urease	-	Lecithinase	-
Esculin hydrolysis	+	MRS agar	Small opaque white colony

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C10 Biochemical test of *L. plantarum* PKWC8-1[*] or TISTR2079[**].

Test	Result	Test	Result	
Aerobe	+	PY base-Dextrose	4.08	+
Anaerobe	+	PY base-Fructose	4.23	+
Hemolysis zone	Beta	PY base-Lactose	4.43	+
Gram stain	Positive rod	PY base-Maltose	4.23	+
Spore	Non spore	PY base-Mannose	4.14	+
Catalase	-	PY base-Mannitol	4.97	+
Oxidase	-	PY base-Salicin	4.03	+
H_2S	-	PY base-Sucrose	4.25	+
Indole	-	PY base-Xylose	5.82	W/+
Motile	-	PY base-Arabinose	4.43	+
Litmus milk	Clot	PY base-Starch	4.61	+
Nitrate	+	Starch hydrolysis	-	
Gelatine	-	Lipase	-	
Urease	-	Lecithinase	-	
Esculin hydrolysis	+	MRS agar	Small opaque white colony	

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C11 Biochemical test of *L. plantarum* PKWD6-10[*] or TISTR 2080[**].

Test	Result	Test	Result	
Aerobe	+	PY base-Dextrose	3.95	+
Anaerobe	+	PY base-Fructose	5.57	+/W
Hemolysis zone	Beta	PY base-Lactose	4.30	+
Gram stain	Positive rod	PY base-Maltose	4.06	+
Spore	Non spore	PY base-Mannose	4.04	+
Catalase	-	PY base-Mannitol	5.01	+
Oxidase	-	PY base-Salicin	4.06	+
H_2S	-	PY base-Sucrose	4.00	+
Indole	-	PY base-Xylose	5.82	W/+
Motile	-	PY base-Arabinose	4.67	+
Litmus milk	Clot	PY base-Starch	4.65	+
Nitrate	-	Starch hydrolysis	-	
Gelatine	-	Lipase	-	
Urease	-	Lecithinase	-	
Esculin hydrolysis	+	MRS agar	Small opaque white colony	

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix Table C12 Biochemical test of *L. plantarum* PKWD7-1* or TISTR 2081**.

Test	Result	Test	Result
Aerobe	+	PY base-Dextrose	3.84 +
Anaerobe	+	PY base-Fructose	5.41 +
Hemolysis zone	Beta	PY base-Lactose	4.27 +
Gram stain	Positive rod	PY base-Maltose	3.89 +
Spore	Non spore	PY base-Mannose	3.91 +
Catalase	-	PY base-Mannitol	4.65 +
Oxidase	-	PY base-Salicin	3.98 +
H_2S	-	PY base-Sucrose	3.92 +
Indole	-	PY base-Xylose	5.89 W/+
Motile	-	PY base-Arabinose	4.25 +
Litmus milk	Clot	PY base-Starch	4.46 +
Nitrate	+	Starch hydrolysis	-
Gelatine	-	Lipase	-
Urease	-	Lecithinase	-
Esculin hydrolysis	+	MRS agar	Small opaque white colony

*: Former identified code number

**: TISTR number obtained from MIRCEN

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Appendix Table C13 Biochemical test of *L. plantarum* PKWD7-1[*] or TISTR 2082 [**].

Test	Result	Test	Result
Aerobe	+	PY base-Dextrose	4.23 +
Anaerobe	+	PY base-Fructose	4.22 +
Hemolysis zone	Beta	PY base-Lactose	4.45 +
Gram stain	Positive rod	PY base-Maltose	4.11 +
Spore	Non spore	PY base-Mannose	4.18 +
Catalase	-	PY base-Mannitol	4.76 +
Oxidase	-	PY base-Salicin	4.09 +
H_2S	-	PY base-Sucrose	4.19 +
Indole	-	PY base-Xylose	5.90 W/+
Motile	-	PY base-Arabinose	4.71 +
Litmus milk	Clot	PY base-Starch	4.54 +
Nitrate	-	Starch hydrolysis	-
Gelatine	-	Lipase	-
Urease	-	Lecithinase	-
Esculin hydrolysis	+	MRS agar	Small opaque white colony

[*]: Former identified code number

[**]: TISTR number obtained from MIRCEN

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Appendix D

New code numbers of *L. plantarum* obtained from the Microbiological Resources Center (MIRCEN), Thailand

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Appendix Table D1 New code numbers of *L. plantarum* obtained from the Microbiological Resources Center (MIRCEN).

Former code number	Change to	TISTR code number
L. plantarum PKWA6-1	→	*L. plantarum* TISTR 2070
L. plantarum PKWA7-1	→	*L. plantarum* TISTR 2071
L. plantarum PKWB6-3	→	*L. plantarum* TISTR 2072
L. plantarum PKWB6-12	→	*L. plantarum* TISTR 2073
L. plantarum PKWB7-1	→	*L. plantarum* TISTR 2074
L. plantarum PKWB7-2	→	*L. plantarum* TISTR 2075
L. plantarum PKWC6-1	→	*L. plantarum* TISTR 2076
L. plantarum PKWC6-9	→	*L. plantarum* TISTR 2077
L. plantarum PKWC7-1	→	*L. plantarum* TISTR 2078
L. plantarum PKWC8-1	→	*L. plantarum* TISTR 2079
L. plantarum PKWD6-10	→	*L. plantarum* TISTR 2080
L. plantarum PKWD7-1	→	*L. plantarum* TISTR 2081
L. plantarum PKWD7-2	→	*L. plantarum* TISTR 2082

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Appendix E

Correlation between cell hydrophobicity of *L. plantarum*
in toluene and xylene solution

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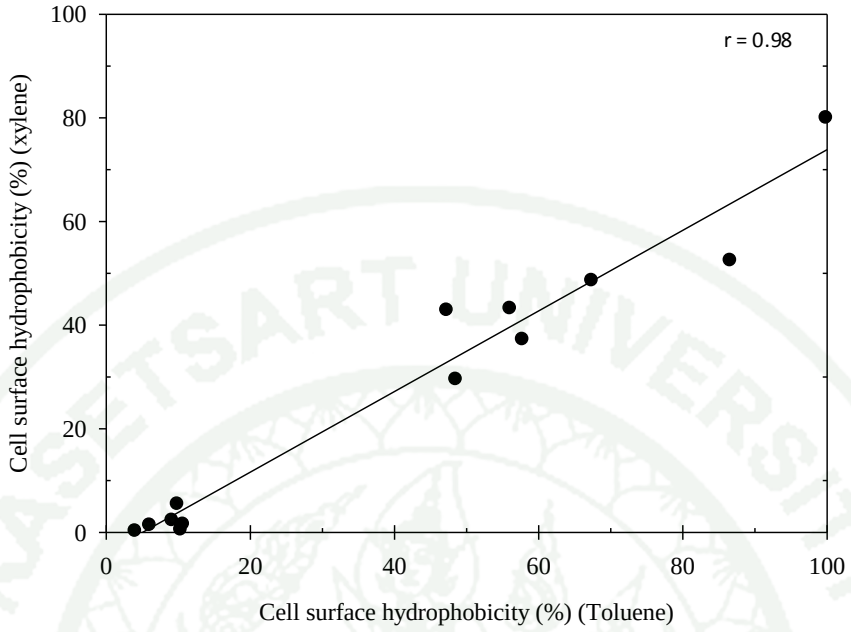

Appendix Figure E1 Correlation between cell hydrophobicity of *L. plantarum* tested in toluene and xylene solution.

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Appendix F

16S rDNA sequencing of *L. plantarum* TISTR 2075 or (PKWB7-2)

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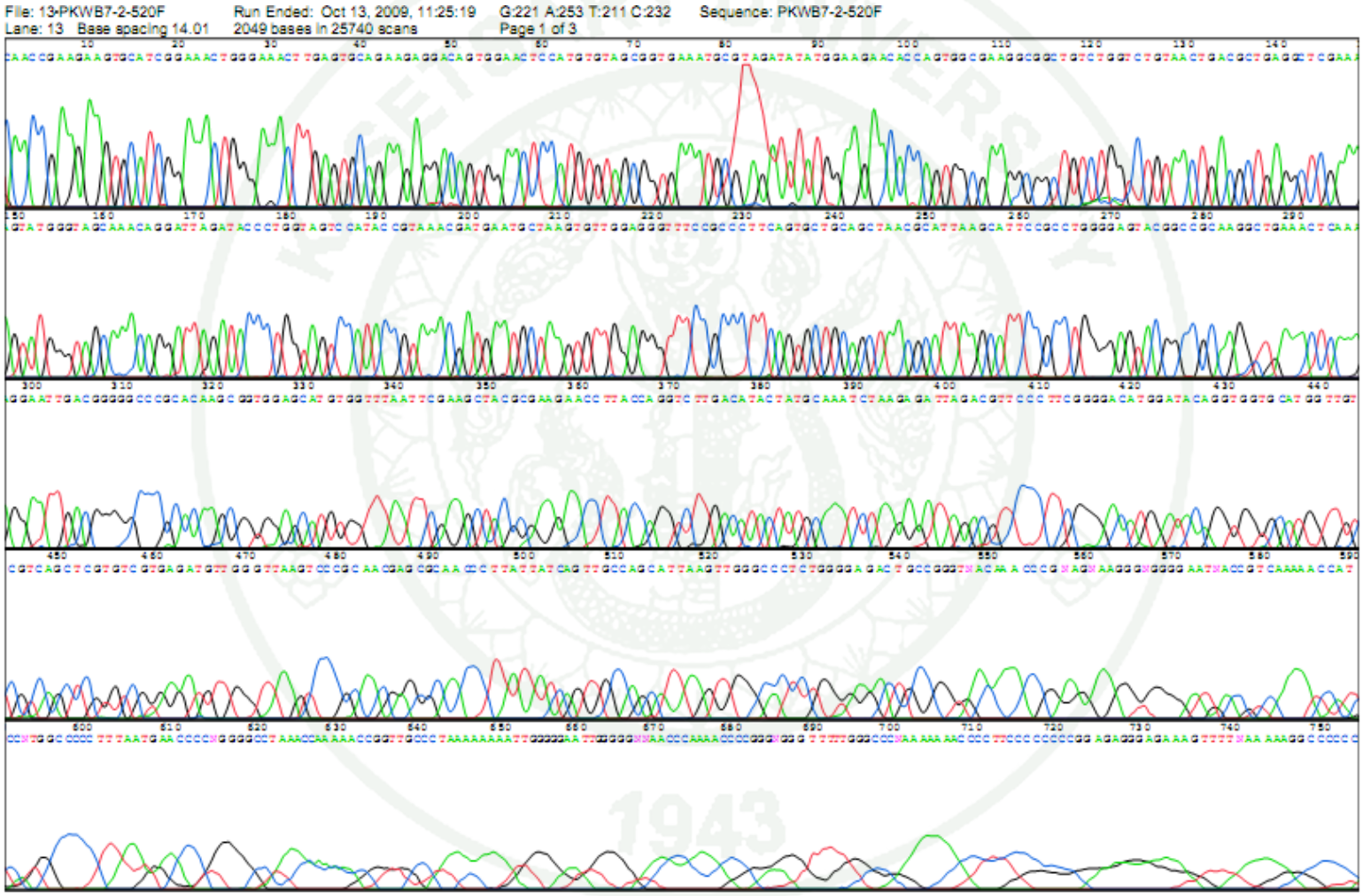

Appendix Figure F1 16S rDNA of *L. plantarum* TISTR 2075 or (PKWB7-2).

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Report of Microbial Identification by partial 16S rDNA sequence analysis

Sample Name : PKWB7-2

500 bp Identification
Homology Search with BLASTn program from NCBI database

Sequences producing significant alignments:		SCORE	E VALUE
GQ917175	*Lactobacillus* sp. CHU-R	902	0.0
FJ751793	*Lactobacillus plantarum* strain DSPV 354T	902	0.0
GQ922602	*Lactobacillus plantarum* strain MBUL90	902	0.0
GQ922601	*Lactobacillus plantarum* strain MBUL77	902	0.0
GQ922598	*Lactobacillus plantarum* strain MBUL91	902	0.0

BLASTN 2.2.22+

Reference:
Stephen F. Altschul, Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

RID: DD7Y8BY6016

Database: All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS,environmental samples or phase 0, 1 or 2 HTGS sequences) 10,122,653 sequences; 29,163,991,683 total letters

Query= PKWB7-2-520F Length=500
>PKWB7-2-520F

CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC

CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC

TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG

GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG

CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA

ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA

CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA

TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC

GCAACGAGCGCAACCCTTAT

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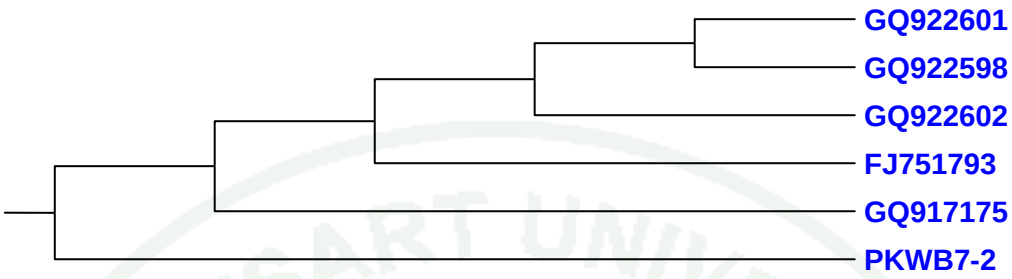
Alignment and Phylogenic tree by MEGA 4

Unweighted pair-group method using arithmetic averages (UPGMA)



>gi|260766932|gb|GQ917175.1| *Lactobacillus* sp. CHU-R 16S ribosomal RNA gene, partial sequence Length=1483

Score = 902 bits (1000), Expect = 0.0
Identities = 500/500 (100%), Gaps = 0/500 (0%)
Strand=Plus/Plus

```
Query  1     CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  612   CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  671

Query  61    CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  672   CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  731

Query  121   TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  732   TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  791

Query  181   GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  240
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  792   GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  851

Query  241   CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  300
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  852   CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  911

Query  301   ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  360
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  912   ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  971

Query  361   CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  420
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972   CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  1031

Query  421   TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  480
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1032  TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  1091

Query  481   GCAACGAGCGCAACCCTTAT  500
             ||||||||||||||||||||
Sbjct  1092  GCAACGAGCGCAACCCTTAT  1111
```

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>gi|260401034|gb|FJ751793.1| *Lactobacillus plantarum* strain DSPV 354T 16S ribosomal RNA gene, partial sequence
Length=1462

Score = 902 bits (1000),Expect = 0.0
Identities = 500/500 (100%), Gaps = 0/500 (0%)
Strand=Plus/Plus

```
Query  1     CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  60
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  602   CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  661

Query  61    CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  662   CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  721

Query  121   TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  180
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  722   TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  781

Query  181   GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  240
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Sbjct  782   GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  841

Query  241   CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  300
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Sbjct  842   CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  901

Query  301   ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  360
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Sbjct  902   ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  961

Query  361   CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  420
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Sbjct  962   CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  1021

Query  421   TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  480
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Sbjct  1022  TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  1081

Query  481   GCAACGAGCGCAACCCTTAT  500
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Sbjct  1082  GCAACGAGCGCAACCCTTAT  1101
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>gi|260533355|gb|GQ922602.2| *Lactobacillus plantarum* strain MBUL90 16S ribosomal RNA gene, partial sequence
Length=1098

Score = 902 bits (1000), Expect = 0.0
Identities = 500/500 (100%), Gaps = 0/500 (0%)
Strand=Plus/Plus

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Query  1    CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  242  CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  301

Query  61   CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  120
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Sbjct  302  CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  361

Query  121  TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  180
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Sbjct  362  TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  421

Query  181  GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  422  GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  481

Query  241  CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  300
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Sbjct  482  CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  541
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Query  301  ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  360
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Sbjct  542  ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  601

Query  361  CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  420
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Sbjct  602  CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  661

Query  421  TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  480
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Sbjct  662  TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  721

Query  481  GCAACGAGCGCAACCCTTAT  500
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Sbjct  722  GCAACGAGCGCAACCCTTAT  741
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>gi|260533354|gb|GQ922601.2| *Lactobacillus plantarum* strain MBUL77 16S ribosomal RNA gene, partial sequence
Length=1492

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 Identities = 500/500 (100%), Gaps = 0/500 (0%)
 Strand=Plus/Plus

Query  1     CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  60
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Sbjct  640   CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  699

Query  61    CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  120
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Sbjct  700   CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  759

Query  121   TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  180
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Sbjct  760   TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  819

Query  181   GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  240
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Sbjct  820   GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  879

Query  241   CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  300
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Sbjct  880   CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  939

Query  301   ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  360
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Sbjct  940   ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  999

Query  361   CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  420
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Sbjct  1000  CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  1059

Query  421   TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  480
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Sbjct  1060  TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  1119

Query  481   GCAACGAGCGCAACCCTTAT  500
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Sbjct  1120  GCAACGAGCGCAACCCTTAT  1139
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>gi|260533351|gb|GQ922598.2| *Lactobacillus plantarum* strain MBUL91 16S ribosomal RNA gene, partial sequence
Length=1461

Score = 902 bits (1000), Expect = 0.0
Identities = 500/500 (100%), Gaps = 0/500 (0%)
Strand=Plus/Plus

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Query  1     CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  60
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Sbjct  631   CAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC  690

Query  61    CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  120
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Sbjct  691   CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTC  750

Query  121   TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  180
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Sbjct  751   TGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTG  810

Query  181   GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  240
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Sbjct  811   GTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTG  870

Query  241   CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  300
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Sbjct  871   CAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGA  930

Query  301   ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  360
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Sbjct  931   ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAA  990

Query  361   CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  420
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Sbjct  991   CCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACA  1050

Query  421   TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  480
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Sbjct  1051  TGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC  1110

Query  481   GCAACGAGCGCAACCCTTAT  500
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LOCUS       GQ917175 1483 bp    DNA     linear   BCT 13-OCT-2009
DEFINITION  Lactobacillus sp. CHU-R 16S ribosomal RNA gene, partial sequence.
ACCESSION   GQ917175
VERSION     GQ917175.1 GI:260766932
KEYWORDS.
SOURCE      Lactobacillus sp. CHU-R
  ORGANISM  Lactobacillus sp. CHU-R
            Bacteria; Firmicutes; Lactobacillales; Lactobacillaceae;
            Lactobacillus.
REFERENCE   1  (bases 1 to 1483)
  AUTHORS   Liao,M.D.
  TITLE     Isolation and taxonomic study of new astaxanthin producing
            bacterium Lactobacillus sp. strain CHU-R
  JOURNAL   Unpublished
REFERENCE   2(bases 1 to 1483)
  AUTHORS   Liao,M.D.
  TITLE     Direct Submission
  JOURNAL   Submitted (15-SEP-2009) College of Resourcese and Environment,
            South China Agricultural University, Number 483, Wushan Road,
            Guangzhou, Guangdong 510642, China
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                     /product="16S ribosomal RNA"
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      361 ccacaatgga cgaaagtctg atggagcaac gccgcgtgag tgaagaaggg tttcggctcg
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      841 cttcagtgct gcagctaacg cattaagcat tccgcctggg gagtacggcc gcaaggctga
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      961 tacgcgaaga accttaccag gtcttgacat actatgcaaa tctaagagat tagacgttcc
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     1141 ctggtgagac tgccggtgac aaaccggagg aaggtgggga tgacgtcaaa tcatcatgcc
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     1261 agagtaagct aatctcttaa agccattctc agttcggatt gtaggctgca actcgcctac
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LOCUS FJ751793 1462 bp DNA linear BCT 07-OCT-2009
DEFINITION *Lactobacillus plantarum* strain DSPV 354T 16S ribosomal RNA gene, partial sequence.
ACCESSION FJ751793
VERSION FJ751793.1 GI:260401034
KEYWORDS.
SOURCE *Lactobacillus plantarum*
ORGANISM *Lactobacillus plantarum*
Bacteria; Firmicutes; Lactobacillales; Lactobacillaceae; Lactobacillus.
REFERENCE 1 (bases 1 to 1462)
AUTHORS Soto,L.P., Frizzo,L.S., Bertozzi,E., Avataneo,E., Sequeira,G.J. and Rosmini,M.R.
TITLE Molecular microbial analysis of *Lactobacillus* strains isolated from the gut of calves for potential probiotic use
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 1462)
AUTHORS Soto,L.P., Frizzo,L.S., Bertozzi,E., Avataneo,E., Sequeira,G.J. and Rosmini,M.R.
TITLE Direct Submission JOURNAL Submitted (15-FEB-2009) Departamento de Salud Publica, Facultad de Ciencias Veterinarias. Universidad Nacional del Litoral, R. P. Kredder 2805, Esperanza, Santa Fe S3080HOF, Argentine
FEATURES Location/Qualifiers
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/isolation_source="calf intestine"
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121 gataacacct ggaaacagat gctaataccg cataacaact tggaccgcat ggtccgagtt
181 tgaaagatgg cttcggctat cacttttgga tggtcccgcg gcgtattagc tagatggtgg
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541 atttattggg cgtaaagcga gcgcaggcgg ttttttaagt ctgatgtgaa agccttcggc
601 tcaaccgaag aagtgcatcg gaaactggga aacttgagtg cagaagagga cagtggaact

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     1381 ccgcccgtca caccatgaga gtttgtaaca cccaaagtcg gtggggaacc tttagaaccg
     1441 ccgctaagtg acaaatgcaa aa

LOCUS       GQ922602            1098 bp    DNA     linear   BCT 05-OCT-2009
DEFINITION  Lactobacillus plantarum strain MBUL90 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   GQ922602
VERSION     GQ922602.2  GI:260533355
KEYWORDS.
SOURCE      Lactobacillus plantarum
  ORGANISM  Lactobacillus plantarum
            Bacteria; Firmicutes; Lactobacillales; Lactobacillaceae;
            Lactobacillus.
REFERENCE   1(bases 1 to 1098)
  AUTHORS   Kumar,R., Prasad,H., Yadav,A.K., Grover,S. and Batish,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2009) Molecular Biology Unit, Dairy
Microbiology
            Division, National Dairy Research Institute, GT Road, Karnal,
            Haryana 132001, India
COMMENT     On Oct 5, 2009 this sequence version replaced gi:259558799.
FEATURES             Location/Qualifiers
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LOCUS       GQ922601          1492 bp    DNA     linear   BCT 05-OCT-2009
DEFINITION  Lactobacillus plantarum strain MBUL77 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   GQ922601
VERSION     GQ922601.2  GI:260533354
KEYWORDS.
SOURCE      Lactobacillus plantarum
  ORGANISM  Lactobacillus plantarum
            Bacteria; Firmicutes; Lactobacillales; Lactobacillaceae;
            Lactobacillus.
REFERENCE   1(bases 1 to 1492)
  AUTHORS   Kumar,R., Prasad,H., Yadav,A.K., Grover,S. and Batish,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2009) Molecular Biology Unit, Dairy
Microbiology
            Division, National Dairy Research Institute, GT Road, Karnal,
            Haryana 132001, India
COMMENT     On Oct 5, 2009 this sequence version replaced gi:259558780.
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  AUTHORS   Kumar,R., Prasad,H., Yadav,A.K., Grover,S. and Batish,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-AUG-2009) Molecular Biology Unit, Dairy
Microbiology
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Appendix G

Lactic acid content in 24 h-culture broth of *L. plantarum* analyzed by HPLC

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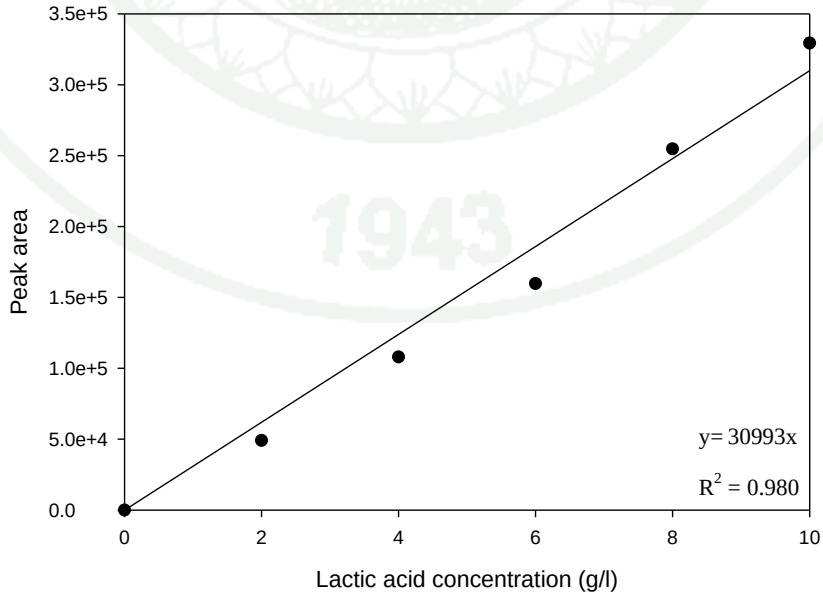
Appendix Table G1 Lactic acid content in 24 h-MRS broth fermented by *L. plantarum* strains.

Strains	Lactic acid content (g/L)
TISTR 2070	17.56
TISTR 2071	19.69
TISTR 2072	21.47
TISTR 2073	19.99
TISTR 2074	20.48
TISTR 2075	18.30
TISTR 2076	11.78
TISTR 2077	11.93
TISTR 2078	11.80
TISTR 2079	11.70
TISTR 2080	10.94
TISTR 2081	11.92
TISTR 2082	12.33



Appendix Figure G1 Standard curve of lactic acid.

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Appendix Table G2 Lactic acid content in 24 h-Job's Tears extract fermented by *L. plantarum* TISTR 2075.

Condition	Glucose concentration (%, w/v)	Lactic acid content (g/L)
Job's Tears extract	0	0.72
	1	2.59
	5	2.68
	10	2.39
	15	2.33
	20	2.18
Job's Tears extract + 1% sesame	0	0.64
	1	4.64
	5	4.56
	10	4.58
	15	4.60
	20	3.01
Job's Tears extract + 2% sesame	0	0.67
	1	4.53
	5	6.66
	10	3.38
	15	3.93
	20	3.35

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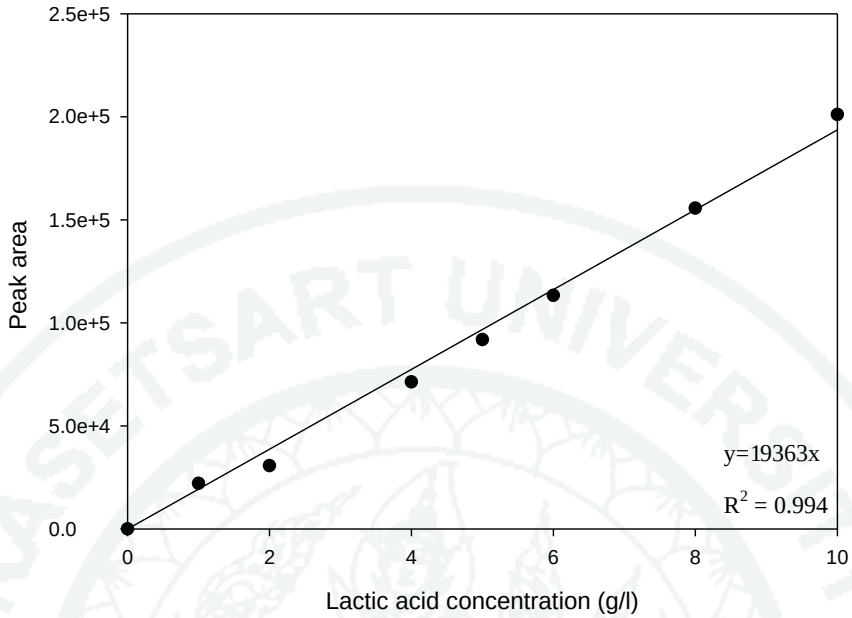

Appendix Figure G2 Standard curve of lactic acid.

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Appendix H

Chemical composition of Job's Tears extract

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Appendix Table H1 Chemical composition of Job's Tears extract.

Characteristics	Values	Method
Moisture (%)	96.48	TMC-02 In house method based on AOAC (2008) 925.45
Protein (%, factor 6.25)	1.53	TMC-03 Kjeldahl method: In house method based on AOAC (2005) 991.20
Fat (%)	0.35	TMC-75 In house method based on AOAC (2005) 989.05
Ash (%)	0.02	TMC-01 In house method based on AOAC (2005) 938.08
Total carbohydrate (%) (by difference; include crude fiber)	1.62	TMC-78 In house method based on AOAC (2005) by Calculation
Total calories (Kcal/100 g)	15.75	TMC-78 In house method based on AOAC (2005) by Calculation
Calories from fat (Kcal/100 g)	3.15	TMC-78 In house method based on AOAC (2005) by Calculation
Starch (%)	8.49	TMC-64 In house method based on AOAC (2006) 948.02
Soluble Dietary Fiber (%)	0.01	TMC-76 In house method based on AOAC (2005) 985.29
Insoluble Dietary Fiber (%)	0.04	TMC-76 In house method based on AOAC (2005) 985.29

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Appendix I

Survival of *L. plantarum* TISTR 2075 grown in soymilk and Job's Tears extracts after spray drying

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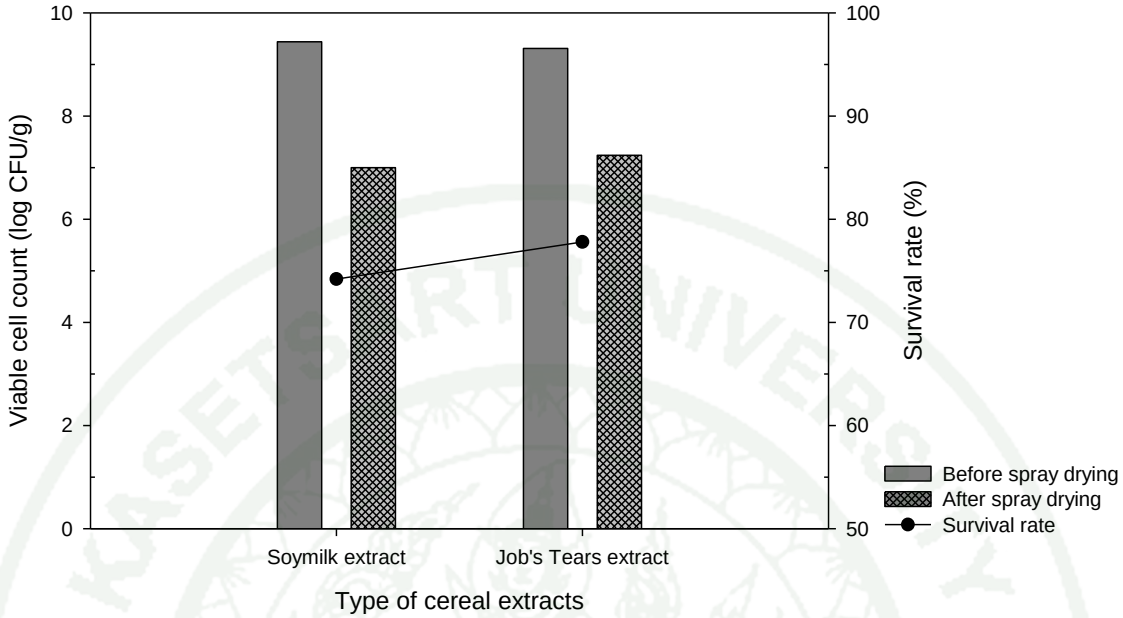

Appendix Figure I1 Survival of *L. plantarum* TISTR 2075 grown in soymilk and Job's Tears extracts after spray drying.

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Appendix J

Viability of *L. plantarum* TISTR 2075 fermented in cereal extracts

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Appendix Table J1 Effect of glucose on the viability of *L. plantarum* TISTR 2075 after 24 h-soymilk fermentation.

Glucose concentration (%)	Viable cell counts (log CFU/mL)		
	Fermented Soymilk	Fermented Soymilk + 1% sesame	Fermented Soymilk + 2% sesame
0% glucose	8.27 [a]	8.30 [b]	8.4 [b]
1% glucose	8.69 [c]	8.66 [a]	8.83 [a]
5% glucose	8.87 [ab]	8.69 [a]	8.64 [ab]
10% glucose	8.96 [a]	8.79 [a]	8.80 [a]
15% glucose	8.79 [bc]	8.60 [a]	8.27 [ab]
20% glucose	8.84 [abc]	8.61 [a]	8.79 [a]

Values with different lowercase letters (a–e) in each column are significantly different by Duncan's multiple range test ($P < 0.05$).

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Appendix Table J2 Effect of glucose on the viability of *L. plantarum* TISTR 2075 after 24 h-Job's Tears extract fermentation.

Glucose concentration (%)	Viable cell counts (log CFU/mL)		
	Fermented Job's Tears extract	Fermented Job's Tears extract + 1% sesame	Fermented Job's Tear extract + 2% sesame
0% glucose	7.73 [b]	8.39 [b]	8.39 [bc]
1% glucose	8.33 [a]	8.67 [a]	8.37 [c]
5% glucose	8.23 [a]	8.64 [ab]	8.48 [abc]
10% glucose	8.36 [a]	8.76 [a]	8.52 [ab]
15% glucose	8.37 [a]	8.63 [ab]	8.54 [a]
20% glucose	8.36 [a]	8.62 [ab]	8.46 [abc]

Values with different lowercase letters (a–c) in each column are significantly different by Duncan's multiple range test ($P < 0.05$).

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Appendix K

Scanning electron microscope of spray-dried powders in different protectants after storage at 4 °C for 2 years

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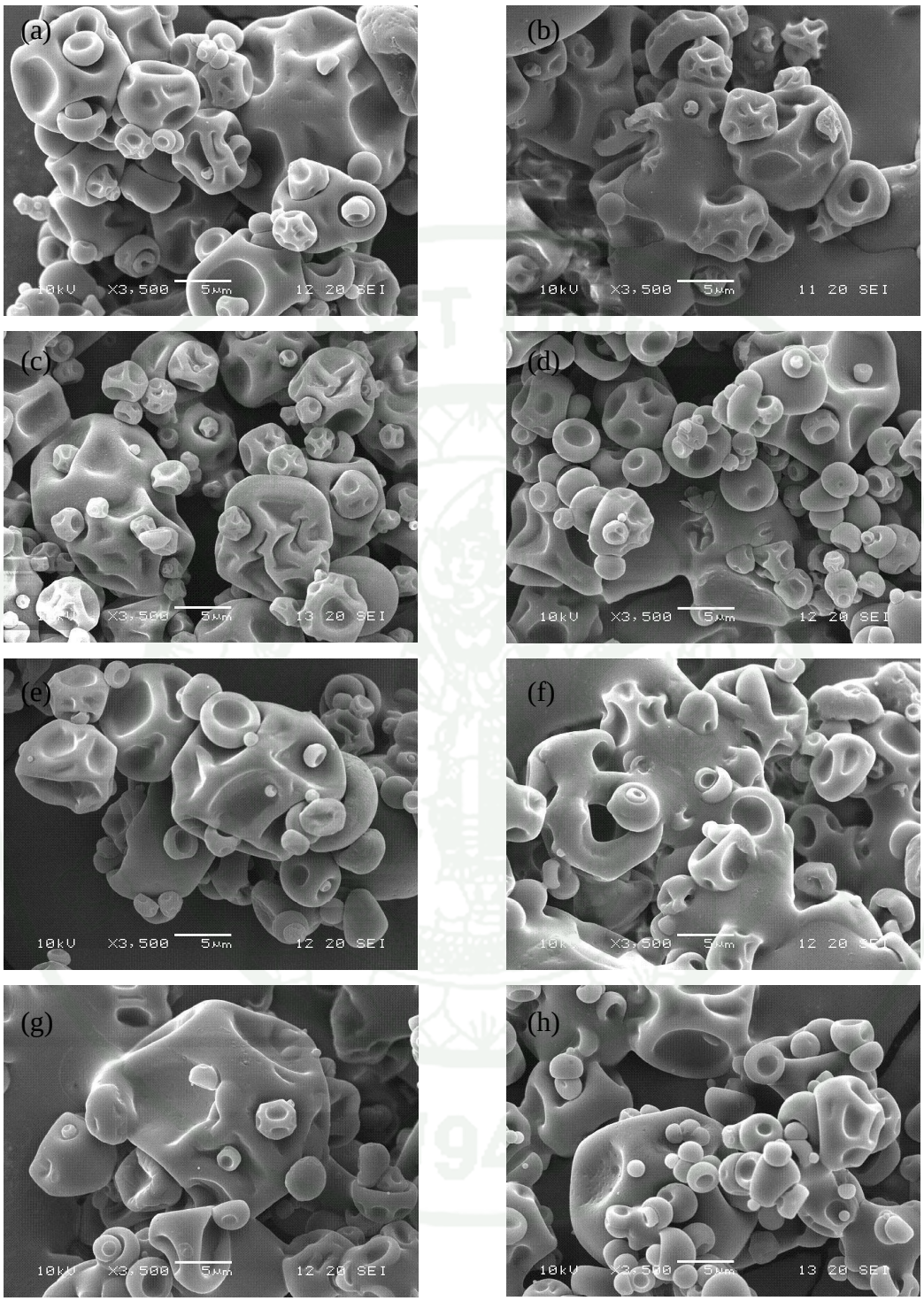

Appendix Figure K1 Scanning electron microscope of spray-dried *L. plantarum* TISTR 2075 in different protectants after storage at 4 °C for 2 years; (a) MD; (b) MD+Tre; (c) MD+Prot; (d) MD+Fib; (e) MD+Asc; (f) MD+Iso; (g) MD+Pal and (h) MD+GA.

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Appendix L

Survival rate of spray-dried *L. plantarum* TISTR 2075 equilibrated under different conditions

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Appendix Table L1 Survival rate of spray-dried *L. plantarum* TISTR 2075 equilibrated under different conditions.

Relative Humidity (%RH)	Temperature (°C)	Survival rate (% ± S.D.)					
		Protectants					
		MD	MD+Prot	MD+Tre	MD+Fib	MD+GA	JTS
11%	4	85.71 ± 0.67	88.80 ± 2.30	88.60 ± 0.79	83.12 ± 1.20	93.26 ± 1.43	94.69 ± 1.84
	25	77.32 ± 1.80	74.72 ± 1.23	76.49 ± 0.71	71.34 ± 0.45	84.47 ± 0.37	88.03 ± 0.46
	40	56.64 ± 3.33	62.91 ± 2.54	66.36 ± 1.11	46.05 ± 8.43	47.30 ± 3.13	76.32 ± 0.60
23%	4	77.20 ± 2.06	82.58 ± 2.12	80.94 ± 0.71	69.21 ± 4.94	85.10 ± 1.12	91.53 ± 3.61
	25	59.45 ± 1.43	66.39 ± 1.74	70.95 ± 1.92	41.27 ± 3.58	44.52 ± 3.88	63.18 ± 2.87
	40	54.87 ± 1.26	35.59 ± 6.71	58.44 ± 1.26	0.00 ± 0.00	0.00 ± 0.00	35.16 ± 0.65
33%	4	73.03 ± 2.50	74.25 ± 2.84	73.44 ± 1.09	59.08 ± 1.76	72.77 ± 0.77	85.94 ± 0.53
	25	55.06 ± 7.47	56.03 ± 3.43	62.11 ± 0.44	36.42 ± 1.51	0.00 ± 0.00	37.91 ± 1.22
	40	47.84 ± 2.21	57.70 ± 2.36	58.97 ± 1.07	0.00 ± 0.00	0.00 ± 0.00	30.35 ± 1.08

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Appendix M

Glass transition temperature of spray drying carriers

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Appendix Table M1 Glass transition temperature (T_g) and moisture content of spray-dried pure carriers.

Carriers	T_g ± S.D. (°C)	a_w	Moisture content (%)
Maltodextrin DE 10	88.30 ± 1.39	0.370 ± 0.000	4.95 ± 0.09
Protein	43.50 ± 1.38	0.316 ± 0.015	4.11 ± 0.48
Trehalose	68.53 ± 1.18	0.522 ± 0.024	9.54 ± 0.29
Fibersol	59.40 ± 0.45	0.281 ± 0.005	5.00 ± 0.06
Acacia Gum	51.33 ± 0.43	0.531 ± 0.006	12.64 ± 0.02
Job's Tears powder	42.12 ± 2.22	0.427 ± 0.002	4.67 ± 0.05

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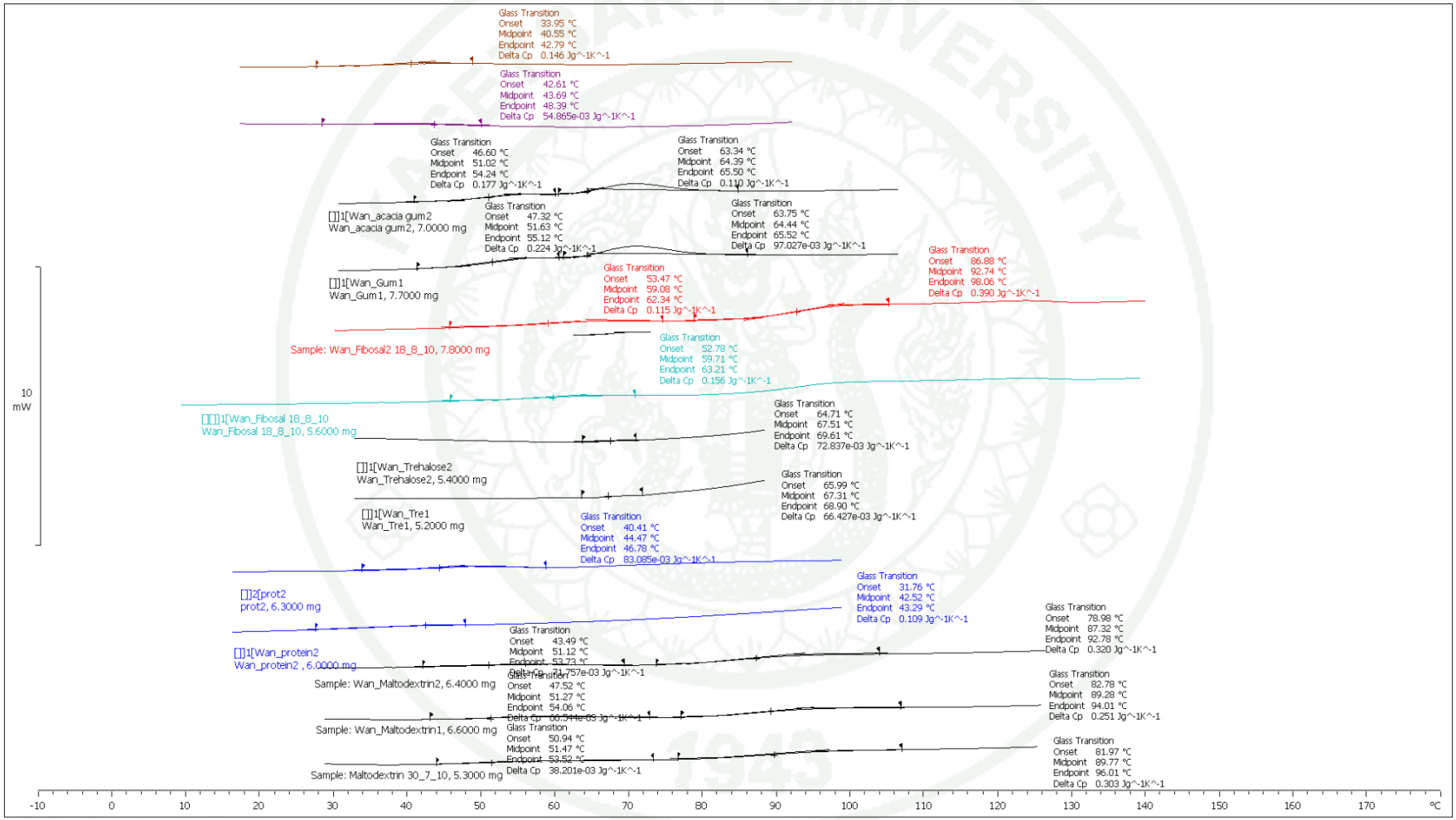

Lab: METTLER STARe SW 9.10

Appendix Figure M1 DSC thermogram of various spray drying carriers

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CURRICULUM VITAE

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SCHOLARSHIP/AWARDS : CHE-PhD-SW: Commission on Higher Education, Ministry of Education, Thailand under the program 'Strategic Scholarships for Frontier Research Network for the Join Ph.D. Program, Thai Doctoral Degree'

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