

**EFFECT OF COLD STRESS ADAPTATION ON VIABILITY OF  
*Lactobacillus casei* Lc-01 IN NON-DAIRY ICE CREAM**

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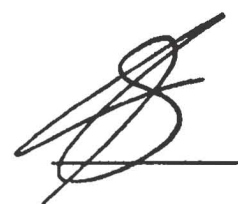
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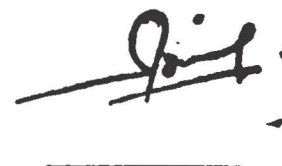
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## ABBREVIATIONS

ADA	American Dietetic Association
ATP	Adenosine triphosphate
ATR	Acid tolerance response
<i>B. spp</i>	<i>Bifidobacterium spp.</i>
C-4	Carbon-4
CAGR	Compound annual growth rate
CDS	Coding sequences
cfu	Colony forming unit
CIPs	Cold-induced proteins
CO <sub>2</sub>	Carbon dioxide
CSPs	Cold-shock proteins
Cu <sup>+</sup>	Copper (II) ion
DNA	Deoxyribonucleic acid
FAO/WHO	Food and Agriculture Organization/World Health Organization
Fe <sup>2+</sup>	Ferum (II) ion
FOS	Fructo-oligosaccharides
GAB	Glutamate decarboxylase
GI	Gastrointestinal
Gpr41	G-protein coupled receptor 41
h	Hour(s)
HCl	Hydrogen chloride
HIPS	High-impact polystyrene
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
kg	Kilogram
LAB	Lactic acid bacteria
lipid-OOH	lipid peroxides
<i>L. spp</i>	<i>Lactobacillus spp.</i>
<i>ldh</i>	Lactate dehydrogenase
LP-MRS	Lithium-propionate
MnKat	Manganese catalase
mg	Milligram
mL	Millilitre
MRS	de Man-Rogosa-Sharpe
NAD <sup>+</sup>	Oxidized nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
O <sub>2</sub> •	Superoxide anion radicals
OH•	Hydroxyl radicals
(p)ppGpp	Phosphate and guanine nucleotide pools
ROS	Reactive oxygen species
SCFAs	Short-chain fatty acids
UK	United Kingdom
USA	United State of America

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## ABSTRACT

Probiotic bacteria *Lactobacillus casei* Lc-01 was incorporated into non-dairy ice cream with stress adaptation treatment at various temperatures and duration prior to frozen storage at -20°C which was found to have obvious effect on their survival. It was found out that the survival rate of probiotic subjected to cold stress adaptation treatment at 25°C for 4 hours increased nearly 50% compared with control. However, at a longer duration (30 hours), cell grows at stress adaptation treatment involved slow cooling from 35°C to 15°C exhibited better survival (97%) after 1 month -20°C frozen storage. Besides, cell subjected to stress adaptation treatment is more metabolically active than non-adapted cell. Probiotic bacteria could act as stabilizer to improve physicochemical properties of non-dairy ice cream. Threonine, tyrosine, cysteine, phenylalanine and isoleucine were only detected in cell subjected to 4 hours cold stress adaptation at 25°C compared with control and the rest of treatments indicate an induction of cold shock proteins. Sensory properties of non-dairy ice cream were overall good if compared with dairy ice cream and can be accepted by consumers. Further studies are needed to evaluate the protection effect of stress adaptation on the probiotic survival in gastrointestinal tract.

## **ABSTRAK**

### **KESAN PENYESUAIAN TEKANAN SEJUK TERHADAP KEGIATAN HIDUP *Lactobacillus casei* Lc-01 DALAM AIS KRIM BUKAN TENUSU**

*Bakteria probiotik Lactobacillus casei Lc-01 telah ditambahkan ke dalam ais krim bukan tenusu dengan rawatan penyesuaian tekanan sejuk (cold stress adaptation) berlainan suhu dan masa sebelum disimpan sejuk beku pada -20°C yang didapati mempengaruhi kegiatan hidup (viability) bakteria. Didapati bahawa ketahanan hidup (survival) bacteria probiotic yang dikenakan rawatan penyesuaian tekanan sejuk pada suhu 25°C selama 4 jam meningkat sebanyak 50% disbanding dengan kawalan. Manakala, untuk rawatan mengambil masa yang lebih panjang (30 jam), sel-sel tumbuh pada rawatan melibatkan suhu pendinginan yang diturunkan secara beransur-ansur daripada 35 °C hingga 15 °C menonjolkan ketahan hidup yang lebih baik (97%) selepas disimpan sejuk beku pada -20°C selama 1 bulan. Selain itu, sel-sel yang dikenakan rawatan penyesuaian tekanan sejuk adalah lebih aktif dari segi metabolik dibanding dengan sel-sel yang tidak mengalami penyesuaian tekanan. Bakteria probiotik boleh bertindak sebagai agen stabil (stabilizer) untuk meningkatkan sifat-sifat fizikimia (physicochemical properties) ais krim bukan tenusu. Threonine, tyrosine, cysteine, phenylalanine dan isoleucine hanya dapat dikesan dalam sel-sel yang dikenakan rawatan penyesuaian tekanan selama 4 jam pada 25°C dibandingkan dengan kawalan dan rawatan lain menunjukkan penampilan cold shock protein. Sifat-sifat sensori ais krim bukan tenusu adalah baik secara keseluruhannya berbanding dengan ais krim tenusu dan dapat diterima oleh konsumen. Penyelidikan mendalam diperlukan untuk mengkaji kesan perlindungan rawatan penyesuaian tekanan sejuk terhadap kegiatan hidup probiotik dalam gastrointestinal tract.*



# CHAPTER 1

## INTRODUCTION

Probiotics are defined as 'live microorganisms that when administrated in adequate amounts confer health benefits to the host' (FAO/WHO, 2002). In recent years, there is a growing interest in incorporating probiotics into various food products especially in dairy industry to develop functional food. American Dietetic Association (ADA, 2009) classifies all foods as functional at some physiological level because they provide nutrients or other substances that furnish energy, sustain growth, or maintain or repair vital processes. However, functional foods move beyond necessity to provide additional health benefits that may reduce disease risk and/or promote optimal health. Functional foods include probiotics, prebiotics and synbiotics (Champagne *et al.*, 2005). Probiotic food is defined as a food product that contains viable probiotic microorganisms in sufficient populations incorporated in a suitable matrix (Homayouni *et al.*, 2008b).

Tremendous health benefits of consuming functional food with probiotics had been proven and described in numerous literatures. These includes reducing gastrointestinal infections (Geier *et al.*, 2006), reduction in serum cholesterol (Shah, 2007), treatment and prevention of rotavirus diarrhea in children (Nagpal *et al.*, 2007), reduction of antibiotic-associated intestinal side-effects (Saad, 2006), inflammatory bowel disease (Bomba *et al.*, 2002), cystic fibrosis, dental caries, irritable bowel syndrome (Santosa *et al.*, 2006), increased relevant specific antibody responses to influenza vaccination (Da Cruz *et al.*, 2009) and improve stress-induced gastrointestinal symptoms (Diop *et al.*, 2008). Meanwhile, studies on functions of probiotics on health are continuously undertaken. This indicates more potential health benefits which are unknown for the time being could be unravelled soon.

According to BCC Research, the global market for probiotic ingredients, supplements and foods was worth \$14.9 billion in 2007 and is expected to reach \$19.6 billion in 2013, a compound annual growth rate (CAGR) of 4.3%. As in

European functional-food market, it is predicted to grow from \$61.7 million (2006) to \$163.5 million in 2013, with particular emphasis on non-dairy food products segment (Yolanda, 2006; McNally, 2007). This is strongly supported by increased availability and promotions of non-dairy based probiotic products include drinks, supplements in the form of tablets, capsules and freeze-dried preparations which utilizing ingredients such as fruits, vegetables, soya and cereals in the market (Yadira & Yoja, 2008).

Ice cream is defined as "a frozen food product containing a minimum of 5% fat and 7.5% milk solids other than fat, which is obtained by heat-treating and subsequently freezing an emulsion of fat, milk solids and sugar (or sweetener), with or without other substances" in UK. Whereas in USA, ice cream must contain at least 10% milk fat and 20% total milk solids, and must weigh a minimum of 0.54 kg l<sup>-1</sup> (Clarke, 2004). According to Malaysia Food Regulations 1985, ice cream shall be made from milk or milk product with milk fat, vegetable fat (not less than 10%), cream, butter or a combination of these and sugar, and may contain other wholesome food. Categories of ice cream include dairy, non-dairy (made with milk proteins and vegetable fat), gelato, frozen yoghurt, milk ice, sorbet, sherbet, water ice and fruit ice.

Among dairy products with live cultures, probiotic ice creams or fermented frozen dessert is gaining popularity (Kailasapathy & Sultana, 2003). It had been shown as innovative food matrix to exhibit great potential as vehicle for delivering probiotic strains in order to give a health boost with added advantage of consumed by all age groups and social levels (Cruz *et al.*, 2009). For incorporation of probiotics into ice cream, several technological parameters and criteria had been emphasized in literatures. These include genus and species of probiotic strain, technological hurdles which can affect survival of probiotics during ice cream processing (ingredients and formulation of ice cream, addition of probiotic culture into ice cream, stability of probiotic during storage period) and effect of probiotic incorporation on sensory properties of ice cream (Homayouni *et al.*, 2008a; Cruz *et al.*, 2009). These criteria henceforth contribute for study of probiotics into non-dairy ice cream.



On the other hand, the efficiency of added probiotic bacteria to exert potential health benefits depends on dose and viability of probiotic throughout product's shelf-life (Homayouni *et al.*, 2008). Therefore, viability of probiotics must be maintained so that certain number can be established in gastrointestinal tract in order to exert positive health effect. As viability of probiotics in delivery system like food matrix depends on strain selected (Vasiljevic & Shah, 2008), they could be selected based on characteristics such as acid, bile salt, oxygen and freezing tolerance (Godward *et al.*, 2000). Whereas concentration of probiotics required for therapeutic effects vary as a function of strain and health effect desired (Champagne, 2005; Cruz *et al.*, 2009). The minimum requirement of viable probiotic number during shelf life of products had been established legislatively in various countries such as  $5 \times 10^8$  CFU/ml (Spain and France),  $10^6$  CFU/ml (Switzerland and Italy),  $10^7$  CFU g<sup>-1</sup> (Japan) and  $10^8$  CFU g<sup>-1</sup> (Portugal) (Biollo *et al.*, 2000). Such legislation is not imposed in many countries especially Asian and developing countries. However, European regulation on health and nutritional claims of functional foods (EC N° 1924/2006) and guidelines for evaluation of probiotics in food published by joint expert committee of the World Health Organization & Food and Agricultural Organization (WHO/FAO, 2002) could be followed for the evaluation of probiotics in food leading to the substantiation of health claims (Yolanda, 2008). Generally, populations of  $10^6$ – $10^7$  CFU/g in the final product are established as therapeutic quantities of probiotic cultures in processed foods. (Cruz *et al.*, 2009)

Viability of probiotics bacteria had been increased through uses of prebiotics (Akin *et al.*, 2007), microencapsulation (Anal & Singh, 2007; Kailasapathy, 2006; Homayouni *et al.*, 2008a), uses of oxygen-impermeable containers, incorporation of micronutrients and stress adaptation (Wang *et al.*, 2005; Homayouni *et al.*, 2008a). It was noted that many bacteria including probiotics, could develop cryotolerance, which is an enhanced capacity to survive exposure to freezing temperature (Panoff *et al.*, 1995; Kim & Dunn, 1997; Lorca & de Valdez, 1999 Derzelle *et al.*, 2003). Two main adaptive physiological responses have been observed in probiotic and lactic acid bacteria towards cold stress. First consists of changes in fatty acid composition of cellular membranes which modulate membrane permeability and second is synthesis of cold shock proteins (Jones *et al.*, 1987; Murata & Wada, 1995; Graumann *et al.*, 1997;



Russell, 1997; Béal *et al.*, 2001; Wang *et al.*, 2005). For first adaptive physiological response, an increase in unsaturated fatty acid content is believed to decrease the solid-to-fluid transition temperature and then, maintain membrane fluidity during cold stress of microorganisms (Russell, 1997; Béal *et al.*, 2001; Wang *et al.*, 2005). On the other hand, synthesis of cold shock proteins in microorganisms which are able to bind to RNA in cooperative manner, and for some of them, function as RNA chaperones, can facilitate the translation process under low positive temperature ((Jones *et al.*, 1987; Graumann *et al.*, 1997).

Although probiotics incorporated in fermented dairy products such as yoghurts and fermented milks have been widely accepted but development of innovative non-dairy probiotic products could serve as an alternative when people refuse to ingest dairy product for particular reason and/or milk products are inaccessible (Heenan *et al.*, 2003; Yadira & Yoja, 2008). Probiotics had been incorporated into fermented and non-fermented dairy ice cream (Homayouni *et al.*, 2008a) but relatively little report had been done on development of probiotic non-dairy ice cream. Besides, drawbacks of dairy products and increased allergenicity towards lactose among populations nowadays promote research in developing of non-dairy products. Hence, research and study on incorporation of probiotics into non-dairy food products would be advantageous and constructive. Though viability of probiotic could be increased through stress adaptation technique, however, little information on the principles underlying this technique in improving viability of probiotics had been reported. Therefore, this study was conducted to investigate the cold adaptation mechanism of probiotics incorporated in non-dairy ice cream during frozen storage.

The specific objectives of this study were:

- 1) To determine the effect of cold adaptation pre-treatment (cold-stress) on the viability of *Lactobacillus casei* Lc-01 during frozen storage (-20°C to -25°C) of non-dairy ice cream.
- 2) To determine the effect of probiotic incorporation on the physicochemical properties of non-dairy ice cream.
- 3) To compare changes of amino acid between cold-stress adapted and non-adapted *Lactobacillus casei* Lc-01 and in non-dairy ice cream.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Probiotics: Lactic acid bacteria

Probiotics of human origin had been extensively incorporated into various food products and most probiotic bacteria belong to the genera *Lactobacillus* and *Bifidobacterium*. Species belonging to genera *Lactococcus*, *Enterococcus*, *Saccharomyces* and *Propionibacterium* are also considered due to their health-promoting effects (Yadira & Yoja, 2008). The genus *Lactobacillus* is the largest group among the lactic acid bacteria (LAB) containing, at present, more than 192 species include subspecies (Lee, 2009; DSMZ-Bacterial Nomenclature Up-to-Date, 2010). The lactobacilli are a broad, morphologically defined group of Gram-positives, nonspore-forming rods or coccobacilli with a G+C content usually below 50mol%. Lactobacilli are clustered in the subdivision of low G+C Gram-positive bacteria, and are included in the phylum *Firmicutes*, class *Bacilli*, order *Lactobacillales*, and family *Lactobacillaceae*. They are strictly fermentative (either homo- or heterofermenters), aerotolerant or anaerobic, aciduric or acidophilic having complex nutritional requirements (Lee, 2009).

Based on phenotypic and biochemical characteristics, lactobacilli were divided into three groups according to the type of sugar fermentation. First group belongs to obligate homofermentative lactobacilli which ferment hexose sugars by glycolysis and produce mainly lactic acid, while obligatory heterofermentative species which utilize the 6-phospho-gluconate/phosphoketolase pathway to produce additional ethanol and carbon dioxide (end products other than lactic acid) belongs to second group. On the other hand, facultative heterofermentative lactobacilli ferment hexoses via glycolysis and pentoses via 6-phosphogluconate/phosphoketolase pathway (Kandler & Weiss, 1901; Lee, 2009). With the development of molecular methods especially analysis of rRNA genes

utilizing PCR technique, the taxonomy of *Lactobacillus* species has changed considerably with increasing knowledge of genomic structure and phylogenetic relationships as shown in Fig 2.1.

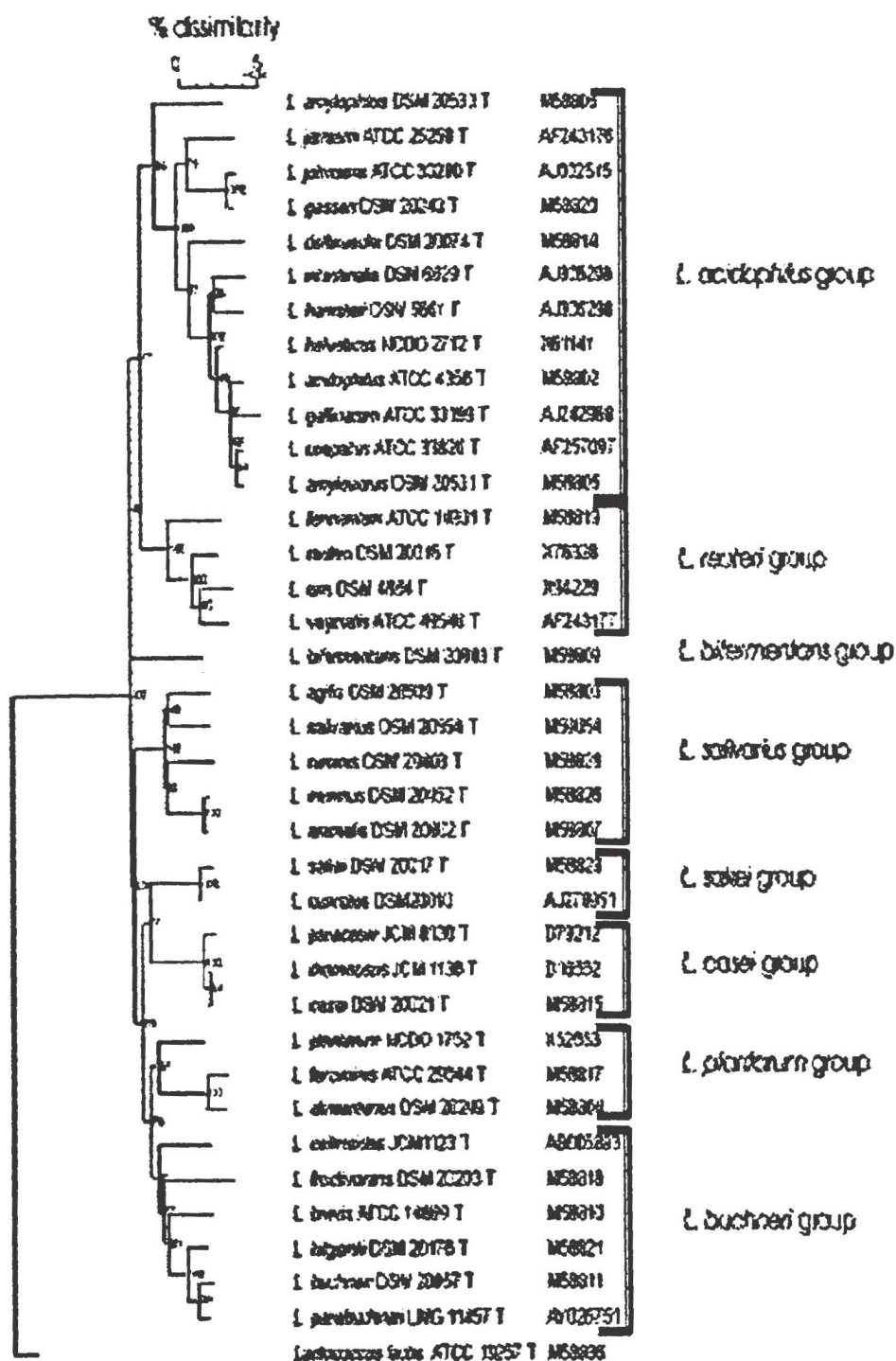


Figure 2.1 Phylogenetic tree showing relationships of the 16S rDNA sequence of type strains of selected *Lactobacillus* species. The tree was constructed by neighbour-joining analysis of a distance matrix from a multiple-sequence alignment. *L. lactis* was used as the outgroup and bootstrap values are indicated at the branch-points (100 trees resampled). Accession numbers are given (adapted from Baele *et al.*, 2002).



Besides, microorganisms of genus *Bifidobacterium* are nonspore-forming, nonmotile, and nonfilamentous rods which display numerous shapes, with slight bends or with a large variety of branching. They are strictly anaerobic with some species are aerotolerant and have fermentative metabolism. Species of genus *Bifidobacterium* form a coherent phylogenetic group and show over 93% similarities to 16s rRNA sequences among them (Satokari *et al.*, 2003; Lee, 2009). One of the main phenotypic features used to identify bifidobacteria at genus level is Fructose-6-phosphate phosphoketolase (F6PPK) activity and F6PPK enzyme as key enzyme of hexose fermentation with lactic and acetic acid produced as metabolic end products. According to DSMZ Bacterial Nomenclature database ([http://www.dsmz.de/microorganisms/bacterial\\_nomenclature](http://www.dsmz.de/microorganisms/bacterial_nomenclature)), the species included in the genus *Bifidobacterium* are 39, namely *B. adolescentis*, *B. angulatum*, *B. animalis*, *B. asteroides*, *B. bifidum*, *B. bombi*, *B. boum*, *B. breve*, *B. catenulatum*, *B. choerinum*, *B. coryneforme*, *B. cuniculi*, *B. denticolens*, *B. dentium*, *B. ballicum*, *B. gallinarum*, *B. globosum*, *B. indicum*, *B. infantis*, *B. inopinatum*, *B. lactis*, *B. longum*, *B. pseudocatenulatum*, *B. pseudolongum*, *B. psychraerophilum*, *B. pullorum*, *B. ruminantium*, *B. saeculare*, *B. scardovii*, *B. subtile*, *B. suis*, *B. thermacidophilum*, *B. thermophilum*. In turn, *B. animalis* is subdivided into two species (*subsp.* *animalis* and *lactis*), *B. longum* into 3 subspecies (*subsp.* *infantis*, *longum* and *suis*). Two subspecies constitutes *B. pseudolongum* (*subsp.* *globosum* and *pseudolongum*) and two constitutes *B. thermacidophilum* (*subsp.* *porcinum* and *thermacidophilum*). *Bifidobacterium* is clustered in the subdivision of high G+C gram-positive bacteria and is included in phylum *Actinobacteria*, class *Actinobacteria*, subclass *Actinobacteridae*, order *Bifidobacteriales*, and family *Bifidobacteriaceae*. *Bifidobacterium*, as currently known, are isolated from very limited number of habitats that is human and animal gastrointestinal tracts (GITs), food, sewage and insect intestine (Satokari *et al.*, 2003; Ventura *et al.*, 2004; Lee, 2009).

A deep deficiency in microbiological quality and labelling of currently marketed probiotic products for human and animal consumption had been revealed through many studies and the incorporation of incorrectly identified probiotic bacteria in functional food products could results in public health implications, by undermining the efficiency of probiotics and by affecting public confidence in functional foods (Lee, 2009). Hence, various molecular techniques

had been utilized to detect and identify bifidobacteria and lactobacilli. These includes amplified ribosomal DNA restriction analysis (ARDRA), randomly amplified polymorphic DNA (RAPD), pulsed field gel electrophoresis (PFGE), ribotyping and community profiling techniques such as PCR coupled to temperature and denaturing gradient gel electrophoresis (PCR-TGGE and PCR-DGGE, respectively) (Satokari *et al.*, 2003). Study of ribosomal rRNA genes (rDNA) is the most common methodology for bifidobacteria identification up to date (Lee, 2009) and a phylogenetic tree of *Bifidobacterium* species type strain based on 16s RNA was shown in Fig 2.2.

Figure 2.2 Phylogenetic tree based on 16S rDNA sequences for each *Bifidobacterium* species type strain. The tree was rooted with *E. combesii* and constructed using the neighbor-joining method. Bootstrap values, expressed as percentages of 1,000 replicons, are given at each branch point where above 50% (adapted from Simpson *et al.*, 2003).

## 2.2 Probiotic food products

For several decades, probiotics have been used in fermented dairy products like yoghurts and fermented milks; however, ways to incorporate these beneficial gastrointestinal bacteria into a much broader range of foods and beverages had been sought by food companies worldwide nowadays (Crittenden, 2009). It is estimated that there are 70 to 80 probiotic-containing products in the world (Champagne *et al.*, 2005; Shah, 2007). Examples of commercial probiotic strains available on market had been summarized in Table 2.1. Recent days, there is an increasing consumer demand for non-dairy based probiotic products which consist of drinks, supplements in the form of tables, capsules and freeze-dried preparations, utilizing ingredients such as fruits, vegetables, soya and cereals (Yadira & Yoja, 2008). For fruits and vegetable category, structural characteristics of fruit and vegetables matrices could be altered through technological advances by modifying food components in a controlled way to develop as carrier of probiotics (Betoret *et al.*, 2003).

Table 2.1 Probiotics and examples of commercial probiotic strains

Probiotic	Examples	Strains (supplier or source)	References
Lactic acid bacteria (LAB)	<i>Lactobacillus acidophilus</i>	LA-1/LA-5 (Chr. Hansen) NCFM (Danisco, Madison, WI, USA) La1 (Nestle, Lausanne, Switzerland) DDS-1 (Nebraska Cultures) SBT-2062 (Snow Brand Milk Products, Tokyo, Japan) LAFTI L10 (DSM, Sdyney, Australia)	Champagne <i>et al.</i> , 2005; Hutkins, 2006
	<i>Lactobacillus delbrueckii subsp. bulgaricus</i>	Lb12	
	<i>Lactobacillus casei</i>	Shirota (Yakult, Tokyo, Japan) DN114001 F19 (Arla Foods, Skanderborgvej, Denmark) Immunitas (Danone)	
	<i>Lactobacillus gasseri</i>	ADH (Danisco, Madison, WI, USA)	
	<i>Lactobacillus johnsonii</i>	KA1 (Nestle, Lausanne, Switzerland) La1	
	<i>Lactobacillus lactis</i>	L1A (Essum AB)	
	<i>Lactobacillus paracasei</i>	CRL 431 (Chr. Hansen) LAFTI L26 (DSM, Sdyney, Australia)	



Table 2.1 continuous

Probiotic	Examples	Strains (supplier or source)	References
	<i>Lactobacillus fermentum</i>	RC-14 (Urex Biotech, London, Canada)	
	<i>Lactobacillus plantarum</i>	299v (Probi, Lund, Sweden) Lp01	
	<i>Lactobacillus rhamnosus</i>	GG (Valio) 271 (Probi AB) GR-1 (Urex Biotech) LB21 (Essum AB)	
	<i>Lactobacillus reuteri</i>	SD2112/MM2 (Biogaia, Stockholm, Sweden)	
	<i>Lactobacillus salivarius</i>	UCC118 (University College, Cork, Ireland)	
Bifido-bacteria	<i>Bifidobacterium adolescentis</i>	ATCC 15703, 94-BIM	Saxelin <i>et al.</i> , 2005; Shah, 2007
	<i>Bifidobacterium bifidus</i>	Bb-11	
	<i>Bifidobacterium breve</i>	Yakult (Yakult, Tokyo, Japan)	
	<i>Bifidobacterium essensis</i>	Danone (Bioactivia)	
	<i>Bifidobacterium infantis</i>	Shirota Immunitass 744 01	
	<i>Bifidobacterium lactis</i>	Bb-12 (Chr. Hansen) Bb-02 Lacti™, B94 (DSM) DR10/HOWARU (Danisco)	
	<i>Bifidobacterium laterosporus</i>	CRL 431	
	<i>Bifidobacterium longum</i>	BB536 (Morina Milk Industry) SBT-2928 (Snow Brand Milk Products, Tokyo, Japan) UCC 35624 (UCCork)	
Yeast	<i>Saccharomyces cerevisiae</i>		
	<i>Saccharomyces boulardii</i>		
Others	<i>Enterococcus faecalis</i>		Champagne <i>et al.</i> , 2005
	<i>Enterococcus faecium</i>		
	<i>Propionibacterium freudenreichii</i>		

### 2.2.1 Non-dairy probiotic products - fruits and vegetables

From the development of probiotic fruit-juice based functional beverages, it was found out that food and vegetables matrices could well support the growth of some lactic acid bacteria strains as shown by reports done (Sheehan *et al.*, 2007; Betoret *et al.*, 2003; Tsen *et al.* 2003; Yoon *et al.*, 2004a; Yoon *et al.*, 2004b; Yoon *et al.*, 2006). Potential of orange, pineapple and cranberry to be developed as probiotic carrier had been studied (Sheehan *et al.*, 2007). Orange and pineapple juice exhibit higher potential than cranberry juice to be developed as probiotic carrier.

By utilizing advanced technology, suitability of fruits and vegetables in delivering probiotics could be greatly increased. Apple matrices had been successfully vacuum impregnated with *Saccharomyces cerevisiae* and *Lactobacillus casei* to be developed as dehydrated probiotic fruit product which could support high viability of *L. casei* (greater than  $10^6$  cfu/g) that is similar to viability observed in commercial dairy products (Betoret *et al.*, 2003). Apart from that, banana had been developed as potential probiotic (*L. acidophilus*) carrier due to prebiotic effect that it exhibits to produce novel synbiotic product by using k-carrageenan cell immobilization technique (Tsen *et al.*, 2003). As cell immobilization normally will be performed on support media (like agar, polyarylamide, calcium pectate gel, chitosan beads, alginate, porous foam glass particles, ceramic beads and gluten pellets), studies utilizing fruits (apple and quince pieces) as novel food-grade support had been proven could enhance survival rate and acid resistance of probiotics (*L. casei*) in extended storage period (Kourkoutas *et al.*, 2005).

For vegetable-based product, suitability of tomato, red-beet and cabbage juice as raw material for production of probiotic juice had been reported (Yoon *et al.*, 2004a; Yoon *et al.*, 2004b; Yoon *et al.*, 2006). Tomato juice can serve as potential probiotic carrier as viable cell counts of probiotics (*Latobacillus acidophilus* LA39, *Lactobacillus plantarum* C3, *Lactobacillus casei* A4, and *Lactobacillus delbrueckii* D7) reached high after 72 hours of fermentation (nearly  $1.0$  to  $9.0 \times 10^9$ /ml) and remained relatively high ( $10^6$  to  $10^8$  CFU/ml) even after 4 weeks of cold storage at 4°C (Yoon *et al.*, 2004a). Potential of red-beet juice to be developed as probiotic non-dairy beverage is encouraging as probiotic cultures were capable of rapidly utilizing beet juice for cell synthesis and lactic acid

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