Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 1406–1416
Food Chemistry
www.elsevier.com/locate/foodchem

Anhydrobiotics: The challenges of drying probiotic cultures

X.C. Meng [a,b], C. Stanton [b,c], G.F. Fitzgerald [c,d], C. Daly [d,e], R.P. Ross [b,c,*]

[a] Key Laboratory of Dairy Science (Ministry of Education) and Food Science & Technology College, Northeast Agricultural University, Harbin, China
[b] Teagasc, Moorepark Food Research Centre, Fermoy Co. Cork, Ireland
[c] Alimentary Pharmabiotic Centre, Cork, Ireland
[d] Microbiology Department, University College Cork, Ireland
[e] Faculty of Food Science & Technology, University College Cork, Ireland

Received 27 October 2006; received in revised form 18 April 2007; accepted 18 April 2007

Abstract

There is accumulating clinical data supporting the role of probiotics in human health particularly in benefiting the immune system, strengthening the mucosal barrier and suppressing intestinal infection. Fermented and unfermented dairy products enriched with probiotic bacteria have developed into one of the most successful categories of functional foods. From a functional ingredient perspective, the generation of these live cultures in dried formats is particularly attractive, however, it does present challenges in terms of retaining probiotic functionality during powder manufacture and storage. Both freeze-dying and spray-drying can be used for manufacture of probiotic powders on a large-scale, however, both approaches expose the cultures to extreme environmental conditions. Methods of production of dried probiotic powders should be such that viability is maintained in the dried powders following manufacture, and storage to ensure that an adequate number of bacteria can be delivered in the final product. This review will focus on how this can be achieved through approaches such as optimizing drying technology, and the drying matrix, and by manipulating probiotic bacteria by classical (microbiological) or genetic approaches.

Keywords: Probiotic; Freeze-drying; Spray-drying; Cell survival; Glass transition temperature

1. Introduction

Probiotics are defined as 'live micro-organisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2001). There is rapidly accumulating clinical evidence that these bacteria can positively affect certain human health conditions, playing an important role in the control of irritable bowel syndrome and inflammatory bowel diseases, suppression of endogenous/exogenous pathogens by normalization of the intestinal microbial composition, alleviation of food allergy symptoms in infants by immunomodulation, lowering serum cholesterol, improving lactose tolerance, and reducing risk factors for colon cancer by metabolic effects (Saarela, Lahteenmaki, Crittenden, Salminen, & Mattila-Sandholm, 2002). Although specific numbers are not mentioned in the definition, high levels of viable micro-organisms are recommended in probiotic foods for efficacy (Knorr, 1998) given that many of the clinical studies use daily doses in excess of 10^9 cfu/day. Consequently, the retention of high viability during drying and storage presents particular challenges and can be regarded as a major bottleneck in commercial probiotic production. This is particularly the case for "technologically sensitive" strains (for example most *Bifidobacterium* species) with the result that most successfully marketed probiotics are usually robust in nature. As a general recommendation and in the absence

* Corresponding author. Address: Teagasc, Moorepark Food Research Centre, Fermoy Co. Cork, Ireland. Tel.: +353 25 42229; fax: +353 25 42340.
E-mail address: paul.ross@teagasc.ie (R.P. Ross).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.04.076

of "dose response" data for many strains, it is suggested that probiotic products should contain at least 10^7 cfu/g (ml) (Ishibashi & Shimura, 1993). Most liquid/frozen probiotic cultures require refrigeration for storage and distribution, thereby adding expense and inconvenience to their widespread use. However these cold-chain requirements may be greatly reduced or eliminated through the use of dry powders, which are potentially superior to liquid/frozen state in their sterility and stability.

Although water is an essential component of life, retention of viability during storage is often enhanced under very low water activity. For example, the inclusion of a silica gel adsorbent with a high water adsorbing capacity in the storage matrix has previously been deemed effective in maintaining viability of dried lactic acid bacterial cells (Sudoma, 1990). Therefore the intention of this review is to discuss how to reduce effects of dehydration processes on probiotic survival. The main topics covered are desiccation technology, including freeze-drying and spray-drying, protectants, cell physiology, which includes stresses, growth phase and medium, storage conditions and rehydration.

2. Desiccation technology

Dehydration is commonly used as a means to stabilize probiotics for their ease of storage, handling, transport and subsequent use in functional food applications. Freeze-drying is the most widespread technique for dehydration of probiotic and dairy cultures, while spray-drying has been applied to the dehydration of a limited number of probiotic cultures.

2.1. Freeze-drying

Freeze-drying has been used to manufacture probiotic powders for decades and is based upon sublimation, occurring in three phases; freezing, primary, and secondary drying. Typically, cells are first frozen at −196 °C and then dried by sublimation under high vacuum (Santivarangkna, Kulozik, & Foerst, 2007). As the processing conditions associated with freeze-drying are milder than spray-drying, higher probiotic survival rates are typically achieved in freeze-dried powders (Wang, Yu, & Chou, 2004). Interestingly, it has been shown that cellular inactivation occurs mostly at the freezing step (Tsvetkov & Brankova, 1983). Indeed, To and Etzel (1997) demonstrated that 60-70% of cells that survived the freezing step can live through the dehydration step. During freezing, the formation of extracellular ice causes an increase in extra-cellular osmolality, so as soon as ice forms outside of the cell in solution, the cell begins to dehydrate. The intracellular and extra-cellular solution concentrations will increase as temperature drops until a eutectic point is reached. There are as such two kinds of freezing methods, i.e. slow freezing and fast freezing. During slow freezing, the process of gradually dehydrating the cell as ice is slowly formed outside the cell leads to extensive cellular damage, while fast freezing can avoid solute effects and excessive cellular shrinkage (Fowler & Toner, 2005). It has been reported that the higher the surface area of the cell, the higher the membrane damage owing to extracellular ice crystal formation during freezing (Fonseca, Beal, & Corrieu, 2000). Consequently, cell size has a strong influence on survival of probiotics during freeze-drying, with small spherical cells such as enterococci being more resistant to freezing and freeze-drying than larger rod shaped lactobacilli (Fonseca et al., 2000).

Removal of bound water from bacterial cells during drying leads to damage of surface proteins, cell wall and the cell membrane. Bound water plays an important role in stabilizing structural and functional integrity of biological macromolecules through different types of weak bonding, including those present on the cell wall and cell membrane. Consequently, water removal during desiccation can lead to destabilisation of the structural integrity of these cellular components, resulting in loss or impairment of function (Brennan, Wanismail, Johnson, & Ray, 1986). It has been proposed that the lipid fraction of the cell membrane is the primary target area for damage during drying, where lipid peroxidation may occur (Brennan et al., 1986; Linders, Wolkers, Hoekstra, & van't Riet, 1997a). In addition, the secondary structures of RNA and DNA destabilize, resulting in reduced efficacy of DNA replication, transcription, and translation (van de Guchte et al., 2002). Therefore, in order to achieve optimum results during the desiccation of probiotics, attention must be strongly focused on approaches to minimize damage to these cellular components.

2.2. Spray-drying

Commercial scale production of freeze-dried cultures is an expensive process with low yields, and as such spray-drying offers an alternative inexpensive approach yielding higher production rates (Zamora, Carretero, & Pares, 2006). The spray-drying process involves the injection of the spray-drying medium at high velocity at temperatures up to 200 °C, which then blasts through a nozzle leading to formation of granules. Consequently, this process results in exposure of the drying medium to high temperatures for a short time, which can be detrimental to the integrity of live bacterial cells. During spray-drying, bacterial cells encounter heat stress, in addition to the other stresses already mentioned during freeze-drying, i.e. dehydration, oxygen exposure and osmotic stress (Brennan et al., 1986; Teixeira, Castro, Mohacsi-Farkas, & Kirby, 1997). The effect of spray-drying on the cell membrane can lead to increased cell permeability which may result in the leakage of intracellular components from the cell into the surrounding environment (Teixeira, Castro, & Kirby, 1995a). The cytoplasmic 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, Castro, Malcata, & Kirby, 1995b; Teixeira

et al., 1997). 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 lamellar phase in which the chains are stiff and fully extended. Furthermore, certain phospholipids undergo a transition from lamellar to hexagonal phase as water is removed (Crowe et al., 1988; Leslie, Israeli, Lighthart, Crowe, & Crowe, 1995).

A number of studies have reported on the performance of a variety of probiotics during spray-drying, and in general, the survival rate of probiotic cultures depends on such factors as the particular probiotic strain used, outlet temperature, and drying medium among others. Using a rifampicin resistant variant of *Lactobacillus paracasei* NFBC 338, it was shown that survival rate of >80% was achievable during spray-drying in RSM (Reconstituted Skim Milk), at outlet temperatures of 85–90 °C (Gardiner et al., 2002), while under similar conditions (outlet temperature of 80 °C), Ananta and Knorr (2003) reported a survival rate of >60% for *L. rhamnosus* GG. It has been shown that different bacterial species vary with respect to spray-drying tolerance, highlighting the importance of strain selection, for example *L. paracasei* NFBC 338 survived significantly better than *L. salivarius* UCC 118 at similar spray-drying conditions (Gardiner et al., 2000), which may be attributed to the greater thermal tolerance of strain *L. paracasei* NFBC 338 compared to *L. salivarius* UCC 118 (Gardiner et al., 2000). When the heat and oxygen tolerance of a number of *Bifidobacterium* species, and the relative performance of selected strains during spray-drying were compared, it was found that closely related species exhibiting superior heat and oxygen tolerance performed best, notably *Bifidobacterium animalis* subsp. *lactis* which survived spray-drying at ~70% or greater in RSM (20% w/v) at an outlet temperature of 85–90 °C (Simpson, Stanton, Fitzgerald, & Ross, 2005). Outlet air temperature is a major processing parameter affecting the number of survivors during spray-drying. For example, Kim and Bhowmik (1990) reported that numbers of *Streptococcus salivarius* subsp. *thermophilus* and *L. debrueckii* subsp. *bulgaricus* decreased with increasing outlet or inlet air temperatures and atomizing air pressure, while similar findings were reported by Gardiner et al., 2000 for both *L. paracasei* NFBC 338 and *L. salivarius* UCC 118. Consequently, improved viability can be achieved by reducing the outlet temperature during spray-drying (Fig. 1), but beyond probiotic viability, powder quality is also influenced by these parameters, with moisture content of ~3.5% being preferred for shelf-stable products (Zayed & Roos, 2004).

3. Protectants

A variety of protectants have been added to the drying media before freeze-drying or spray-drying to protect the viability of probiotics during dehydration, including skim milk powder, whey protein, trehalose, glycerol, betaine, adonitol, sucrose, glucose, lactose and polymers such as dextran and polyethylene glycol (Hubalek, 2003; Morgan, Herman, White, & Vesey, 2006). Compatible cryoprotectants may be added to media prior to fermentation to assist in the adaptation of probiotics to the environment (Capela, Hay, & Shah, 2006). As compatible cryoprotectants accumulate within the cells, the osmotic difference between the internal and external environments is reduced (Kets, Teunissen, & de Bont, 1996a). The use of gum acacia in the spray-drying medium resulted in enhanced probiotic survival of *L. paracasei* NFBC 338, which displayed 10-fold greater survival than control cells (20% RSM) when grown in a mixture of RSM (10% w/v) and gum acacia (10% w/v) prior to spray-drying at air outlet temperature of 100–105 °C (Desmond, Ross, O'Callaghan, Fitzgerald, & Stanton, 2002). RSM appears to be a very suitable media for efficacious spray-drying of probiotic cultures (Ananta, Volkert, & Knorr, 2005; Corcoran, Ross, Fitzgerald,

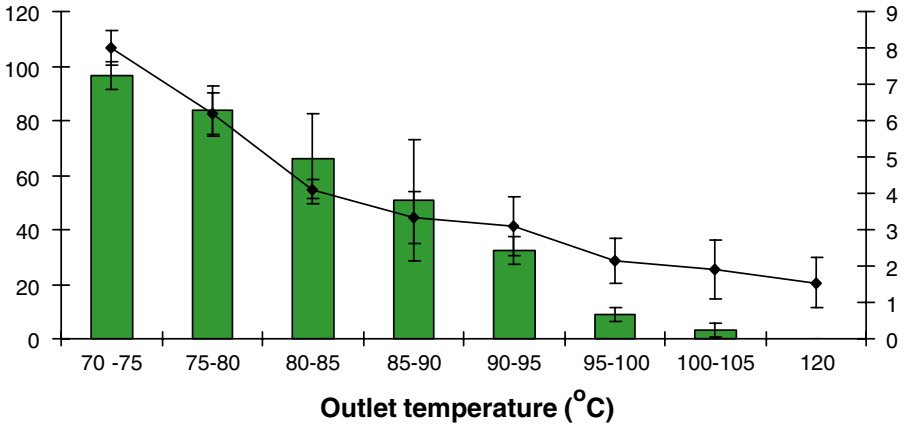

Fig. 1. Survival of *Lactobacillus paracasei* NFBC 338 during spray drying in 20% (wt/vol) RSM supplemented with 0.5% (wt/vol) yeast extract at different air outlet temperatures (bar graph). The line shows the moisture contents of the resulting powders. The air inlet temperature was maintained at 170 °C. The results are means based on data from duplicate spray-drying trials, and standard deviations are indicated by vertical bars (Gardiner et al., 2000).

& Stanton, 2004; Desmond et al., 2002) as skim milk protein can prevent cellular injury by stabilizing cell membrane constituents (Castro, Teixeira, & Kirby, 1995). Furthermore, it may form a protective coating on the cell wall proteins, while calcium in milk increases survival after dehydration (King & Su, 1993). Corcoran et al. (2004) reported that the inclusion of the prebiotics polydextrose and inulin in the spray-drying medium (RSM) did not enhance viability during spray-drying or powder storage. On the other hand, survival of *L. helveticus* during vacuum drying was improved by the addition of 1% sorbitol (Santivarangkna, Kulozik, & Foerst, 2006). It is well documented that carbohydrates have protective effects for probiotic bacteria during freeze-drying, given that these cryprotectants can raise the glass-phase transition temperature, and therefore viable cells can reach the glassy phase without nucleating intracellular ice (Fowler & Toner, 2005). It also has been demonstrated that trehalose is an effective cryoprotectant during freezing and freeze-drying, enabling higher survival of *L. acidophilus* (Conrad, Miller, Cielenski, & de Pablo, 2000), due to the remarkably high glass transition temperature (T_g) of trehalose, and the strong ion–dipole interactions and hydrogen bonding between trehalose and the biomolecule (Patist & Zoerb, 2005). In a recent study in our laboratory, we compared the protective effects of a series of disaccharides on *L. rhamnosus* GG survival during freeze-drying and storage, and found that trehalose, trehalose/lactose and lactose/maltose were the most efficacious disaccharides during both freezing and freeze-drying (unpublished). Compatible solutes have also proven beneficial in probiotic viability protection in acidic environments. For example, the presence of 19.4 mM glucose resulted in up to 6-$\log_{10}$-enhanced survival following 90 min of exposure to simulated gastric juice at pH 2.0 compared with the control (Corcoran, Stanton, Fitzgerald, & Ross, 2005). In this study, it was reported that the presence of glucose resulted in the provision of ATP to F0F1-ATPase via glycolysis, thus enabling proton exclusion from the cell and thereby enhancing survival in simulated gastric environments.

4. Cell physiology

The importance of cell physiology to the successful drying of probiotics has been demonstrated in a number of studies, and in this respect, several factors have been proposed which have an influence on the survival of probiotic bacteria during dehydration, e.g. stress treatment, growth phase of the probiotic culture prior to dehydration, growth media and genetic modification.

4.1. Application of mild stress prior to dehydration

The application of sublethal stress to enhance the stress responses prior to dehydration has been demonstrated as one feasible approach, ensuring high viability of bacterial cultures and retention of physiological activity during dehydration (de Urraza & de Antoni, 1997; Desmond et al., 2002; Kim, Khunajakr, & Dunn, 1998; Lorca & de Valdez, 1998; Teixeira et al., 1995a). It has been demonstrated that bacteria respond to changes in their immediate surroundings by a metabolic reprogramming which leads to a cellular state of enhanced resistance (Pichereau, Hartke, & Auffray, 2000). Resistance encoded by defence systems can be divided into two classes. The first comprises a specific system induced by a sublethal dose of a chemical or physical stress (e.g. heat shock), that permits survival against a challenge dose of the same agent (Desmond, Stanton, Fitzgerald, Collins, & Ross, 2001; Gouesbet, Jan, & Boyaval, 2001; Pichereau et al., 2000). The second class of resistance comprises more general systems which prepare cells to survive against very different environmental stresses, without the need for cultures to have had prior exposure to that stress (Desmond et al., 2001; Gouesbet et al., 2001; Pichereau et al., 2000). This mechanism is known as cross-protection (Kim, Perl, Park, Tandianus, & Dunn, 2001). Indeed, pre-adaptation with heat or salt led to improved heat tolerance of probiotics during spray-drying. For example, *L. paracasei* NFBC 338, pre-adapted by exposure to 0.3 M NaCl, was significantly more resistant to heat stress associated with spray-drying (outlet temperatures between 95 °C and 100 °C) than non-adapted control cells ($33.46 \pm 2.3\%$ *versus* $8.27 \pm 4.42\%$ survival, respectively) (Desmond et al., 2001). Although not as efficient as the homologous stress, the levels of cross-protection were in the order heat $\sim$ salt $>$ hydrogen peroxide $>$ bile (Stanton et al., 2002).

In the case of the first type of resistance mentioned, mild heat treatments can lead to adaptation of the cell membrane by increasing the saturation and the length of the fatty acids in order to maintain optimal fluidity of the membrane and activity of intrinsic proteins (Russell & Fukanaga, 1990). The beneficial effect of heat stress in the conservation of probiotics can also be explained by the production of heat shock proteins (HSPs) which promote the correct folding of nascent polypeptides, assembly of protein complexes, degradation and translocation of proteins (Bukau & Horwich, 1998; De Angelis & Gobbetti, 2004). The two major groups of chaperone proteins are the 70-kDa DnaK family and the 60-kDa GroE family which function as chaperone machines (Georgopoulos & Welch, 1993). The components of the DnaK chaperone typically consist of DnaK, DnaJ and GrpE, while that of GroE is composed of GroEL and GroES (De Angelis & Gobbetti, 2004). Desmond, Fitzgerald, Stanton, and Ross (2004) reported that chaperone protein GroEL was among the most strongly expressed proteins in the cell under heat adaptation conditions. Viability of the heat-adapted *L. paracasei* NFBC 338 in RSM was enhanced 18-fold during spray-drying at outlet temperatures of 95–105 °C (Desmond et al., 2001). Heat shock induction of the *groESL* chaperone in *L. johnsonii* also provided protection against freezing (Walker, Girgis, & Klaenhammer, 1999). The increased cytoplasmic contentrations of GroES and

GroEL favored cell viability and metabolic activity during and after starter preparation using freezing, lyophilisation or spray-drying (De Angelis & Gobbetti, 2004). Furthermore, pressure pre-treatment has also been shown to increase heat resistance of probiotics. For example, it has been demonstrated that incubation of *L. rhamnosus* GG at elevated pressure of 100 MPa for 5–10 min prior to exposure to lethal heat at 60 °C led to increased heat resistance as compared to untreated controls (Ananta & Knorr, 2003).

4.2. Growth phase

When grown in batch culture, the growth of bacterial cultures occurs during four distinct phases, i.e. log, lag, stationary and death phases. It is known that the stress responses of bacterial cultures vary depending on the growth phase. Indeed, bacteria that enter into stationary phase develop a general stress resistance and are 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 (Brashears & Gilliland, 1995; Lorca & de Valdez, 1999; Morgan et al., 2006; van de Guchte et al., 2002). Therefore, the optimal growth phase for dehydration survival is the stationary phase. For example, it was reported that stationary phase cells of *L. rhamnosus* yielded the highest recovery rates after drying (31–50% survival), whereas early log-phase cells exhibited only 14% survival, and lag phase cells showed the highest susceptibility, with only a 2% cell survival under similar conditions of drying (Corcoran et al., 2004). However, in earlier studies on the freeze-drying of lactic acid bacteria, late-logarithmic (Champagne, Mondou, Raymond, & Roy, 1996) or (early) stationary (Carvalho et al., 2004a; Wang et al., 2004; Zayed & Roos, 2004) phase cells were commonly used. On the other hand, Saarela et al. (2004) reported that no differences were observed between the freeze and drying and storage stability performance of *B. animalis* subsp. *lactis* cells grown to a late-logarithmic growth phase (15 h) or to an early stationary phase (22 h). Interestingly, Carvalho et al. (2003a) reported that starvation of stationary phase *L. bulgaricus* cultures resulted in improved resistance during storage in the dried state.

The final pH of the growth media of the probiotic culture also influences the survival during desiccation. It was reported that the highest viability (~80% survival) was obtained following freeze-drying, when *L. reuteri* cells were grown at pH 5 and harvested after 2.5 h in the stationary phase (Palmfeldt & Hahn-Hagerdal, 2000). Moreover, the cells obtained under non-controlled pH (4.5) conditions were more resistant to heat stress, spray-drying and storage in the dried state than those from culture under controlled pH (6.5) (Silva et al., 2005). This phenomenon may be related to acid shock/adaptation, which can alter the physiological state of bacterial cells leading to enhanced synthesis of heat shock proteins, and hence improvement of resistance to drying, as described above. Silva et al. (2005) confirmed that the higher resistance of *L. delbrueckii* subsp. *bulgaricus* grown under non-controlled pH correlated with the enhanced production of heat shock proteins. However, Linders et al. (1997a) found that pH control during growth of *L. plantarum* cells resulted in a higher residual activity after drying in a fluidized bed (37% survival) compared to growth without pH control (19% survival).

4.3. Growth media

The composition of the growth media is a contributing factor to the survival rate of probiotic cultures during drying, and in this respect, the importance of the presence of carbohydrates has been demonstrated. For example, the growth of *L. delbrueckii* subsp. *bulgaricus* in the presence of sugars, such as lactose, sucrose and trehalose, or chemical cryoprotectants, such as, glycerol, showed that cells can be adapted to freezing and thawing by an osmotic stress (Panoff, Thammavongs, & Gueguen, 2000). The survival of *L. sakei* following spray-drying was enhanced when cells were grown in the presence of sucrose (Ferreira et al., 2005). Tymczyszyn, Gomez-Zavaglia, and Disalvo (2007) reported the difference in the effectiveness of lactose, sucrose and trehalose in the recovery of *L. delbrueckii* subsp. *bulgaricus* following drying, when grown at different water activities. Indeed, it has been demonstrated that the preservation of dehydrated bacteria with sucrose, after growing them in a low water activity medium (MRS-sucrose), appears to be as efficient as dehydration with trehalose. The reason for this is most likely that the bacterial cells adapted to the low water activity medium. It has also been reported that the lowest decrease in viability after freeze-drying was obtained when *L. bulgaricus* was grown in the presence of mannose, compared to fructose, lactose or glucose (Carvalho et al., 2003b; Carvalho et al., 2004a). Other sugars, such as fructose and sorbitol also provided better protection than glucose, the standard growth media carbohydrate (Carvalho et al., 2004a). The mechanism for the protection of sugars in the growth media is likely that growth in the presence of various sugar substrates produces cells with distinct morphological and physiological traits, thus reflecting distinct resistances to the various stress treatments tested (Carvalho et al., 2004b). Studies have shown that metabolites such as mannitol, sorbitol and glutamate which in most cases remain inside the cell may be responsible for the distinct survival behaviour during dehydration (Wisselink, Weusthuis, Eggink, Hugenholtz, & Grobben, 2002), and the formation of these metabolites depends on the carbon sources in the growth media (Kets, Galinski, de Wit, de Bont, & Heipieper, 1996b).

A number of other factors, including the presence of sodium chloride in the growth media and medium

concentration also have effects on the survival of probiotic cultures following dehydration. Linders et al. (1997a) demonstrated that the presence of 1 mol l^{-1} or 1.25 mol l^{-1} NaCl during growth of *L. plantarum* resulted in a decreased residual activity after drying in the fluidized bed. In addition, the residual activity after drying was higher for cells grown in diluted MRS than for cells grown in enriched MRS, despite differences in betaine and carnitine accumulation. However the effect was not significant.

4.4. Genetic-modification of probiotic strains

Advances in genomics and proteomics have led to the identification of genes involved in *Lactobacillus* stress responses, such as the molecular chaperone *groESL* and *dnaK* (heat stress) (Prasad, McJarrow, & Gopal, 2003; Schmidt, Hertel, & Hammes, 1999; Walker et al., 1999), and enhanced viability of probiotic cultures during dehydration has been obtained by over-expression of the genes encoding various stress inducible proteins. Walker et al. (1999) revealed that features of the *groESL* operon are shared between various lactic acid bacteria, notably other *Lactobacillus* species and *Lactococcus lactis*. Two-dimensional polyacrylamide gel electrophoresis also revealed that GroEL expression in probiotic *L. paracasei* NFBC 338 was increased under heat adaptation conditions (52 °C for 15 min) (Desmond et al., 2004). Furthermore, Corcoran, Ross, Fitzgerald, Dockery, and Stanton (2006) demonstrated that GroESL over-expression in *L. paracasei* NFBC 338 resulted in improved performances during spray-drying and freeze-drying but did not contribute to enhanced survival of probiotic cultures during storage in the powder form. Furthermore, using transmission electron microscopy (TEM), it was apparent that the probiotic lactobacilli present in both spray-dried and freeze-dried powders were in close contact (Fig. 2) (Corcoran et al., 2006). Because an increased ability to accumulate betaine via BetL can improve the ability of an organism to prevail in diverse, stressful environments, the nisin-controlled expression system was used to direct the heterologous expression of the

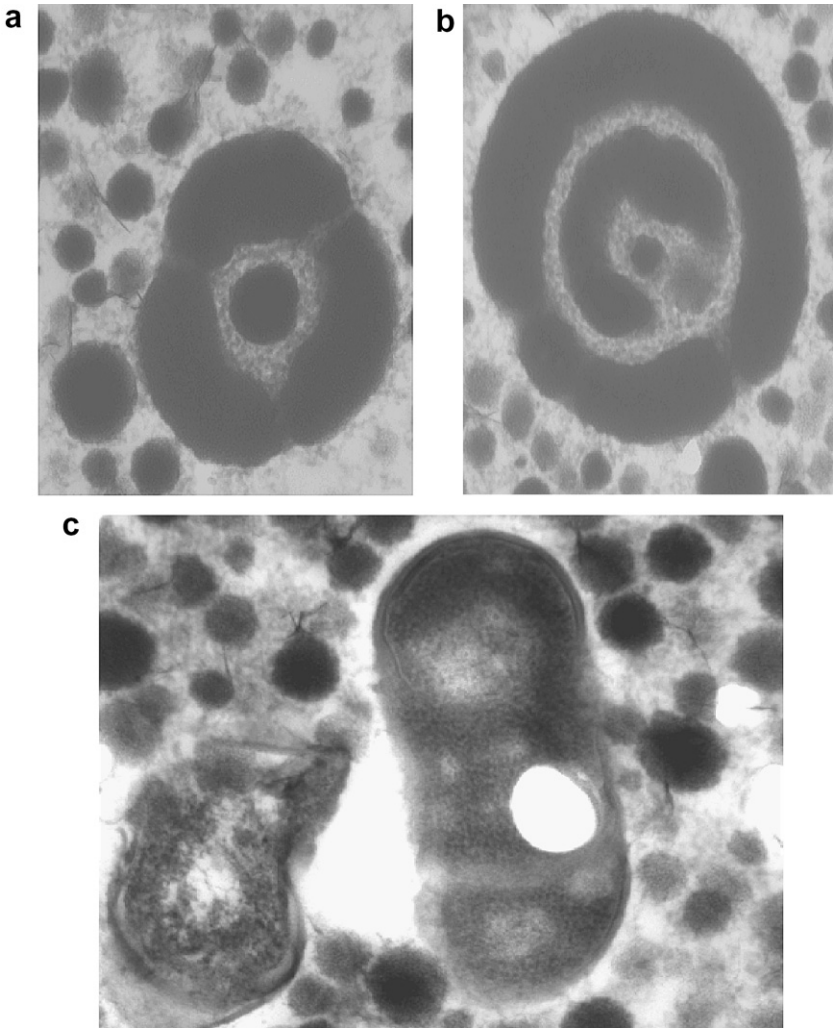

Fig. 2. (a) TEM image of spray-dried log-phase *L. paracasei* NFBC 338, overproducing GroESL (60,000×). (b) TEM image of spray-dried log-phase *L. paracasei* NFBC 338, control (60,000×). (c) TEM image of freeze-dried log-phase *L. paracasei* NFBC 338, control (60,000×) (Corcoran et al., 2006).

listerial betaine uptake system BetL in the probiotic strain *L. salivarius* UCC118 (Sheehan, Sleator, Fitzgerald, & Hill, 2006). Following nisin induction, strains expressing *betL* exhibited a significant increase in resistance to several stresses, including elevated osmo-, cryo-, baro-, and chill tolerance, as well as increased resistance to spray and freeze-drying. The percent survival of UCC118-BetL$^+$ during freeze-drying was 36%, compared to 18% for UCC118-BetL$^-$ (Sheehan et al., 2006). The *tre* locus also plays an important role in freezing and lyophilisation of *L. acidophilus* (Duong, Barrangou, Russell, & Klaenhammer, 2006). Analysis of the *L. acidophilus* NCFM genome by Duong et al. (2006) revealed a putative trehalose utilization locus consisting of a transcriptional regulator, *treR*; a trehalose phosphoenolpyruvate transferase system (PTS) transporter, *treB*; and a trehalose-6-phosphate hydrolase, *treC*. These workers demonstrated that disruption of both the trehalose transporter and hydrolase genes abolished the ability of *L. acidophilus* NCFM to grow on trehalose and reduced the survival *of L. acidophilus* NCFM when subjected to repeated cycles of freezing and thawing in the presence of trehalose, indicating that not only is the internalization of trehalose important, but also its subsequent hydrolysis is a contributing factor.

5. Storage and rehydration

5.1. Storage

The storage conditions i.e. storage temperature, moisture content of powders, relative humidity, powder composition, oxygen content, exposure to light and storage materials, have significant influences on the survival of probiotics in dried powders, and the correct storage conditions are essential to maintain viable populations of freeze and spray-dried probiotic bacteria.

Viability of probiotic bacteria during powder storage is inversely related to storage temperature (Gardiner et al., 2000; Mary, Moschetto, & Tailliez, 1993; Silva, Carvalho, Teixeira, & Gibbs, 2002; Teixeira et al., 1995b). Bruno and Shah (2003) demonstrated that a temperature maintained at −18 °C was optimum for the long-term storage of freeze-dried probiotics to maximize viability of bifidobacteria, whereas a storage temperature of 20 °C was unsuitable, resulting in significant reductions in viable counts. Furthermore, Simpson et al. (2005) reported that there was a significant decline in viability of a number of bifidobacteria species when spray-dried in a skimmed milk-based carrier and stored at 15 °C and 25 °C. The moisture content of probiotic powders is a critical factor influencing shelf-life stability of the live bacteria. Work conducted in our laboratory has shown that viability of freeze-dried probiotics in skim milk is inversely related to relative vapour pressure (RVP), with 11.4% RVP yielding highest viability during storage at room temperature (unpublished). Zayed and Roos (2004) also demonstrated that the amount of water remaining after drying affects not only the viability of bacteria, as determined immediately after the process, but also the rate of loss of viability during subsequent storage. Indeed, the optimum moisture content for storage of freeze-dried *L. salivarius* subsp. *salivarius* was reported to range from 2.8% to 5.6% (Zayed & Roos, 2004).

The carrier used during the spray-drying and freeze-drying of probiotics is known to have an influence on storage stability. Ananta et al. (2005) evaluated the effect of a spray-dried carrier on protection of *L. rhamnosus* GG at 25 °C and 37 °C storage conditions, and found that the protection capacity decreased in the order RSM > RSM/Polydextrose > RSM/Raftilose®P95. Moreover, stability of *L. rhamnosus* GG during long-term storage was impaired by partial substitution of skim milk with either of the prebiotic substances evaluated. Similar findings were previously reported by Corcoran et al. (2004) for spray-dried probiotic lactobacilli. These data justify the suitability of skim milk as a medium for the large-scale production of shelf-stable spray-dried probiotic bacteria. Some studies have shown that the presence of disaccharides can stabilize the cell membrane during both freezing and storage (Carvalho et al., 2002; Conrad et al., 2000; Crowe et al., 1988). For example, it has been proposed that sorbitol prevents membrane damage by interaction with the membrane (Linders, de Jong, Meerdink, & Vantriet, 1997b), and stabilizes protein functionality and structure (Yoo & Lee, 1993). Control of the phase transition temperature in membranes of dry cells is an important factor determining desiccation tolerance of live probiotics, in addition to control of free radical activity (Linders et al., 1997a). It has been suggested that the decrease of viability during storage at high temperatures and/or relative humidity for sugar-containing products has been related to their glass transition temperature (Vega & Roos, 2006). The reason for this is that sugars are likely to form highly viscous glasses at room temperature when they are dehydrated, and the improved storage of anhydrobiotes and liposomes has been associated with the presence of a glassy state. Our data demonstrated that high viability of freeze-dried *L. rhamnosus* GG powders in trehalose, lactose/trehalose and lactose/maltose related to their high glass transition temperature (unpublished). However, Carvalho et al. (2002) and Linders et al. (1997a) demonstrated that sorbitol was the most effective protectant for *L. plantarum* and *L. rhamnosus* during storage, while in contrast, the superior glass former, trehalose was not an effective protectant.

The impairment of viability during storage is related to oxidation of membrane lipids (Teixeira, Castro, & Kirby, 1996). Unsaturated acyl lipids such as oleic acid can not be considered as stable food constituents during food storage, as the presence of one or more allyl groups within the fatty acid molecule are readily oxidized to hydroperoxides. Moreover, products of lipid peroxidation have been shown to induce DNA damage in a model system (Akasaka, 1986; Inouye, 1984) and in bacteria (Marnett et al., 1985). Therefore, to minimize oxidation and thereby optimize probiotic

viability during storage, the presence of antioxidants (Teixeira et al., 1995b), in combination with storage under vacuum with controlled water activity should be effective.

Microencapsulation of various bacterial cultures including probiotics has also been used for extending their storage life (Krasaekoopt, Bhandari, & Deeth, 2003). Several methods of micro-encapsulation of probiotic bacteria have been reported and include extrusion technique, emulsion technique, cross-linking with cationic polymers, coating with other polymers, mixing with starch and incorporation of additives (Krasaekoopt et al., 2003). Song, Cho, and Park (2003), Kim, Kamara, Good, and Enders (1988) Koo, Cho, Huh, Baek, and Park (2001) observed that both non-encapsulated and encapsulated cells stored at 4 °C had comparable stability, while encapsulation provided a greater degree of protection against increased storage temperature. O'Riordan, Andrews, Buckle, and Conway (2001) prepared microencapsulated *Bifidobacterium* PL-1 with starch by spray-drying, however the starch-coated cells did not display any enhanced viability compared with free PL1 cells when exposed to acid conditions for 6 h or in two dry food preparations over 20 days storage at ambient temperature (19–24 °C). Hence, the efficiency of micro-encapsulation of probiotics depends on the encapsulating materials and techniques of micro-encapsulation.

5.2. Rehydration

Rehydration of probiotic powders is the final critical step for the revival of cells after dehydration. The reconstitution process in water can be divided into four steps: wetting, submersion, dispersion and dissolving (Freudig, Hogekamp, & Schubert, 1999). Among these steps, wetting of the particles is very often the reconstitution controlling step (Vega & Roos, 2006). The rehydration solution itself (in terms of osmolarity, pH and nutritional energy source), as well as the rehydration conditions (in terms of rehydration temperature and volume) may significantly affect the rate of recovery to the viable state, and thus influence survival rates (Carvalho et al., 2004b). For optimum results, it is recommended to dry the cells at the stationary phase of growth and to use slow rehydration procedures (Teixeira et al., 1995a). Poirier, Marechal, Richard, and Gervais (1999) have hypothesized that increased cell recovery of *Saccharomyces cerevisiae* is achieved when the dried cells were rehydrated slowly (7–16 days) under controlled conditions, rather than immediate rehydration. Studies have shown that the rehydration media also can influence probiotic recovery significantly. For example, complex media such as 10% (w/v) RSM and PTM media (1.5% (w/v) peptone), 1% (w/v) tryptone and 0.5% (w/v) meat extract as well as a 10% (w/v) sucrose solutions were found to produce significantly higher bacterial cell recovery than media such as phosphate buffer, sodium glutamate and water (Costa, Usall, Teixido, Garcia, & Vinas, 2000). Some studies have indicated that the same rehydration solution as used for cryopreservation results in increased viability (Abadias, Teixido, Usall, Benabarre, & Vinas, 2001; Ray, Jezeski, & Busta, 1971). The reason for this is that such a solution provides a high osmotic pressure environment which could control the rate of hydration, and thus avoid osmotic shock. The temperature of rehydration of freeze-dried and spray-dried probiotics also influences cell recovery. For example, Ray et al. (1971) found that rehydration at 15–25 °C produced the highest numbers of recovered *Salmonella anatum* cells, compared to 35 °C and 45 °C where the cell recovery was lower.

6. Conclusions

Fermented and unfermented dairy products enriched with probiotic bacteria have developed into one of the most successful categories of functional foods, and in this respect, the availability of dried probiotic powders for convenient functional food applications limits their widespread application. Methods of production of dried probiotic powders should be such that adequate numbers of viable probiotic bacteria are maintained in the dried powder following manufacture, and also retention/stability of probiotic properties should be ensured throughout shelf-life. Both freeze-drying and spray-drying can be used for manufacture of probiotic powders on a large scale, but these processes result in exposure of the live probiotic bacteria to a variety of stresses, such as heat, cold, oxygen and osmotic stresses, leading to impaired functionality and loss of viability during drying and storage. For optimum results, it is therefore important to consider a variety of factors, including the selection of the particular probiotic strain, the condition of the culture entering the dryer, the use of protectants and environmental water activity. Furthermore, genomics and proteomics have provided further insights into the cellular processes that can help live probiotic bacteria overcome the stresses associated with desiccation.

Acknowledgements

This work was funded by SFI funds, and in part by the Irish Government under the National Development Plan 2000–2006. Prof. Meng was a recipient of the State Scholarship Fund from China Scholarship Council.

References

Abadias, M., Teixido, N., Usall, J., Benabarre, A., & Vinas, I. (2001). Viability, efficacy, and storage stability of freeze-dried biocontrol agent *Candida sake* using different protective and rehydration media. *Journal of Food Protection, 64*, 856–861.

Akasaka, S. (1986). Inactivation of transforming activity of plasmid DNA by lipid peroxidation. *Biochimica et Biophysica Acta, 867*, 201–208.

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

Ananta, E., & Knorr, D. (2003). Pressure-induced thermotolerance of *Lactobacillus rhamnosus* GG. *Food Research International, 36*, 991–997.

Brashears, M. M., & Gilliland, S. E. (1995). Survival during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* cells as influenced by the growth phase. *Journal of Dairy Science, 78*, 2326–2335.

Brennan, M., Wanismail, B., Johnson, M. C., & Ray, B. (1986). Cellular damage in dried *Lactobacillus acidophilus*. *Journal of Food Protection, 49*, 47–53.

Bruno, F. A., & Shah, N. P. (2003). Viability of two freeze-dried strains of *Bifidobacterium* and commercial preparations at various temperatures during prolonged storage. *Food Microbiology and Safety, 68*, 2336–2339.

Bukau, B., & Horwich, A. L. (1998). The Hsp70 and Hsp60 chaperone machines. *Cell, 92*, 351–366.

Capela, P., Hay, T. K. C., & Shah, N. P. (2006). Effect of cryoprotectants, prebiotics and microencapsulation on survival of probiotic organisms in yoghurt and freeze-dried yoghurt. *Food Research International, 39*, 203–211.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2002). Survival of freeze-dried *Lactobacillus plantarum* and *Lactobacillus rhamnosus* during storage in the presence of protectants. *Biotechnology Letters, 24*, 1587–1591.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2003a). Effect of various factors upon thermotolerance and survival during storage of freeze-dried *Lactobacillus delbrueckii* ssp. *bulgaricus*. *Journal of Food Science, 68*, 2538–2541.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2003b). Effect of various growth media upon survival during storage of freeze-dried *Enterococcus faecalis* and *Enterococcus durans*. *Journal of Applied Microbiology, 94*, 947–952.

Carvalho, A. S., Silva, J., Ho, P., Teixeira, P., Malcata, F. X., & Gibbs, P. (2004a). Effects of various sugars added to growth and drying media upon thermotolerance and survival throughout storage of freeze-dried *Lactobacillus delbrueckii* ssp. *bulgaricus*. *Biotechnology Progress, 20*, 248–254.

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

Castro, H., Teixeira, P., & Kirby, R. (1995). Storage of lyophilized cultures of *Lactobacillus bulgaricus* under different relative humidities and atmospheres. *Applied Microbiology and Biotechnology, 44*, 172–176.

Champagne, C. P., Mondou, F., Raymond, Y., & Roy, D. (1996). Effect of polymers and storage temperature on the stability of freeze-dried lactic acid bacteria. *Food Research International, 29*, 555–562.

Conrad, P. B., Miller, D. P., Cielenski, P. R., & de Pablo, J. J. (2000). Stabilization and preservation of *Lactobacillus acidophilus* in saccharide matrices. *Cryobiology, 41*, 17–24.

Corcoran, B. M., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2004). Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. *Journal of Applied Microbiology, 96*, 1024–1039.

Corcoran, B. M., Stanton, C., Fitzgerald, G. F., & Ross, R. P. (2005). Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. *Applied and Environmental Microbiology, 71*, 3060–3067.

Corcoran, B. M., Ross, R. P., Fitzgerald, G. F., Dockery, P., & Stanton, C. (2006). Enhanced survival of GroESL-overproducing *Lactobacillus paracasei* NFBC 338 under stressful conditions induced by drying. *Applied and Environmental Microbiology, 72*, 5104–5107.

Costa, E., Usall, J., Teixido, N., Garcia, N., & Vinas, I. (2000). Effect of protective agents, rehydration media and initial cell concentration on viability of *Pantoea agglomerans* strain CPA-2 subjected to freeze-drying. *Journal of Applied Microbiology, 89*, 793–800.

Crowe, J. H., Crowe, L. M., Carpenter, J. F., Rudolph, A. S., Wistrom, C. A., Spargo, B. J., et al. (1988). Interactions of sugars with membranes. *Biochimica et Biophysica Acta, 947*, 367–384.

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

de Urraza, P., & de Antoni, G. (1997). Induced crytolerance of *Lactobacillus delbrueckii* subsp. *bulgaricus* LBB by preincubation at suboptimal tempreatures with fermentable sugar. *Cryobiology, 35*, 159–164.

Desmond, C., Fitzgerald, G. F., Stanton, C., & Ross, R. P. (2004). Improved stress tolerance of GroESL-overproducing *Lactococcus lactis* and probiotic Lactobacillus paracasei NFBC 338. *Applied Environmental Microbiology, 70*, 5929–5936.

Desmond, C., Stanton, C., Fitzgerald, G. F., Collins, K., & Ross, R. P. (2001). Environmental adaptation of probiotic lactobacilli towards improvement of performance during spray-drying. *International Dairy Journal, 11*, 801–808.

Desmond, C., Ross, R. P., O'Callaghan, E., Fitzgerald, G., & Stanton, C. (2002). Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. *Journal of Applied Microbiology, 93*, 1003–1011.

Duong, T., Barrangou, R., Russell, W. M., & Klaenhammer, T. R. (2006). Characterization of the *tre* locus and analysis of trehalose cryoprotection in *Lactobacillus acidophilus* NCFM. *Applied and Environmental Microbiology, 72*, 1218–1225.

FAO/WHO (2001). *Evaluation of health and nutritional properties of powder milk with live lactic acid bacteria: Report from FAO/WHO expert consultation* (pp. 1–4).

Ferreira, V., Soares, V., Santos, C., Silva, J., Gibbs, P. A., & Teixeira, P. (2005). Survival of *Lactobacillus sakei* during heating, drying and storage in the dried state when growth has occurred in the presence of sucrose or monosodium glutamate. *Biotechnology Letters, 27*, 249–252.

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

Fowler, A., & Toner, M. (2005). Cryo-injury and biopreservation. *Annals of New York Academy of Sciences, 1066*, 119–135.

Freudig, B., Hogekamp, S., & Schubert, H. (1999). Dispersion of powders in liquids in a stirred vessel. *Chemical Engineering and Processing, 38*, 525–532.

Gardiner, G. E., O'Sullivan, E., Kelly, J., Auty, M. A., Fitzgerald, G. F., Collins, J. K., et al. (2000). Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray-drying. *Applied and Environmental Microbiology, 66*, 2605–2612.

Gardiner, G. E., Bouchier, P., O'Sullivan, E., Kelly, J., Collins, J. K., Fitzgerald, G. F., et al. (2002). A spray-dried culture for probiotic Cheddar cheese manufacture. *International Dairy Journal, 12*, 749–756.

Georgopoulos, C., & Welch, W. J. (1993). Role of the major heat shock proteins as molecular chaperones. *Annual Review of Cell Biology, 9*, 601–634.

Gouesbet, G., Jan, G., & Boyaval, P. (2001). *Lactobacillus delbrueckii* ssp. *bulgaricus* thermotolerance. *Le Lait, 81*, 301–309.

Hubalek, Z. (2003). Protectants used in the cryopreservation of microorganisms. *Cryobiology, 46*, 205–229.

Inouye, S. (1984). Site-specific cleavage of double-strand DNA by hydroperoxide of linoleic acid. *FEBS Letters, 172*, 231–234.

Ishibashi, N., & Shimura, S. (1993). Bifidobacteria: Research and development in Japan. *Food Technology, 46*, 126–135.

Kets, E., Teunissen, P., & de Bont, J. (1996a). Effect of compatible solutes on survival of lactic acid bacteria subjected to drying. *Applied and Environmental Microbiology, 62*, 259–261.

Kets, E. P., Galinski, E. A., de Wit, M., de Bont, J. A., & Heipieper, H. J. (1996b). Mannitol, a novel bacterial compatible solute in *Pseudomonas putida* S12. *Journal of Bacteriology, 178*, 6665–6670.

Kim, H. S., Kamara, B. J., Good, I. C., & Enders, G. L. (1988). Method for the preparation of stable microencapsulated lactic acid bacteria. *Journal of Industrial Microbiology, 3*, 253–257.

Kim, S. S., & Bhowmik, S. R. (1990). Survival of lactic acid bacteria during spray-drying of plain yoghurt. *Journal of Food Science, 55*, 1010–2008.

Kim, W.-S., Perl, L., Park, J.-H., Tandianus, J. E., & Dunn, N. W. (2001). Assessment of stress response of the probiotic *Lactobacillus acidophilus*. *Current Microbiology, 43*, 346–350.

Kim, W. S., Khunajakr, N., & Dunn, N. W. (1998). Effect of cold shock on protein synthesis and on cryotolerance of cells frozen for long periods in *Lactococcus lactis*. *Cryobiology, 37*, 86–91.

King, V., & Su, J. (1993). Dehydration of *Lactobacillus acidophilus*. *Process Biochemistry, 28*, 47–52.

Knorr, D. (1998). Technology aspects related to microorganisms in functional foods. *Trends in Food Science Technology, 9*, 295–306.

Koo, S. M., Cho, Y. H., Huh, C. S., Baek, Y. J., & Park, J. (2001). Improvement of the stability of *Lactobacillus casi* YIT 9018 by microencapsulation using alginate and chitosan. *Journal of Microbiology and Biotechnology, 11*, 376–383.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal, 13*, 3–13.

Leslie, S. B., Israeli, E., Lighthart, B., Crowe, J. H., & Crowe, L. M. (1995). Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Applied and Environmental Microbiology, 61*, 3592–3597.

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

Linders, L. J. M., de Jong, G. I. W., Meerdink, G., & Vantriet, K. (1997b). Carbohydrates and the dehydration inactivation of *Lactobacillus plantarum*: The role of moisture distribution and water activity. *Journal of Food Engineering, 31*, 237–250.

Lorca, G. L., & de Valdez, G. F. (1998). Temperature adaptation and cryotolerance in *Lactobacillus acidophilus*. *Biotechnology Letters, 20*, 847–849.

Lorca, G. L., & de Valdez, G. F. (1999). The effect of suboptimal growth temperature and growth phase on resistance of *Lactobacillus acidophilus* to environmental stress. *Cryobiology, 39*, 144–149.

Marnett, L. J., Hurd, H. K., Hollstein, M. C., Levin, D. E., Esterbauer, H., & Ames, B. N. (1985). Naturally occurring carbonyl compounds are mutagens in *Salmonella tester* strain TA 104. *Mutation Research, 148*, 25–34.

Mary, P., Moschetto, N., & Tailliez, R. (1993). Production and survival during storage of spray-dried *Bradyrhizobium japonicum* cell concentrates. *Journal of Applied Bacteriology, 74*, 340–344.

Morgan, C. A., Herman, N., White, P. A., & Vesey, G. (2006). Preservation of micro-organisms by drying: A review. *Journal of Microbiological Methods, 66*, 183–193.

O'Riordan, K., Andrews, D., Buckle, K., & Conway, P. (2001). Evaluation of microencapsulation of a *Bifidobacterium* strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology, 91*, 1059–1066.

Palmfeldt, J., & Hahn-Hagerdal, B. (2000). Influence of culture pH on survival of *Lactobacillus reuteri* subjected to freeze-drying. *International Journal of Food Microbiology, 55*, 235–238.

Panoff, J. M., Thammavongs, B., & Gueguen, M. (2000). Cryoprotectants lead to phenotypic adaptation to freeze–thaw stress in *Lactobacillus delbrueckii* ssp. bulgaricus CIP 101027T. *Cryobiology, 40*, 264–269.

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

Pichereau, V., Hartke, A., & Auffray, Y. (2000). Starvation and osmotic stress induced multiresistances:Influence of extracellular compounds. *International Journal of Food Microbiology, 55*, 19–25.

Poirier, I., Marechal, P. A., Richard, S., & Gervais, P. (1999). *Saccharomyces cerevisiae* viability is strongly dependant on rehydration kinetics and the temperature of dried cells. *Journal of Applied Microbiology, 86*, 87–92.

Prasad, J., McJarrow, P., & Gopal, P. (2003). Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. *Applied and Environmental Microbiology, 69*, 917–925.

Ray, B., Jezeski, J. J., & Busta, F. F. (1971). Effect of rehydration on recovery, repair, and growth of injured freeze-dried *Salmonella anatum*. *Applied Microbiology, 22*, 184–189.

Russell, N., & Fukanaga, N. (1990). A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria. *FEMS Microbiol Review, 75*, 171–182.

Saarela, M., Lahteenmaki, L., Crittenden, R., Salminen, S., & Mattila-Sandholm, T. (2002). Gut bacteria and health foods—the European perspective. *International Journal of Food Microbiology, 78*, 99–117.

Saarela, M., Rantala, M., Hallamaa, K., Nohynek, L., Virkajarvi, I., & Matto, J. (2004). Stationary-phase acid and heat treatments for improvement of the viability of probiotic lactobacilli and bifidobacteria. *Journal of Applied Microbiology, 96*, 1205–1214.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2006). Effect of carbohydrates on the survival of *Lactobacillus helveticus* during vacuum drying. *Letters in Applied Microbiology, 42*, 271–276.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2007). Alternative drying processes for the industrial preservation of lactic acid starter cultures. *Biotechnology Progress, 23*, 302–315.

Schmidt, G., Hertel, C., & Hammes, W. P. (1999). Molecular characterisation of the dnaK operon of *Lactobacillus sakei* LTH681. *Systematic and Applied Microbiology, 22*, 321–328.

Sheehan, V. M., Sleator, R. D., Fitzgerald, G. F., & Hill, C. (2006). Heterologous expression of BetL, a betaine uptake system, enhances the stress tolerance of *Lactobacillus salivarius* UCC118. *Applied and Environmental Microbiology, 72*, 2170–2177.

Silva, J., Carvalho, A. S., Teixeira, P., & Gibbs, P. A. (2002). Bacteriocin production by spray-dried lactic acid bacteria. *Letters in Applied Microbiology, 34*, 77–81.

Silva, J., Carvalho, A. S., Ferreira, R., Vitorino, R., Amado, F., Domingues, P., et al. (2005). Effect of the pH of growth on the survival of *Lactobacillus delbrueckii* subsp. *bulgaricus* to stress conditions during spray-drying. *Journal of Applied Microbiology, 98*, 775–782.

Simpson, P. J., Stanton, C., Fitzgerald, G. F., & Ross, R. P. (2005). Intrinsic tolerance of *Bifidobacterium* species to heat and oxygen and survival following spray-drying and storage. *Journal of Applied Microbiology, 99*, 493–501.

Song, S.-H., Cho, Y.-H., & Park, J. (2003). Microencapsulation of *Lactobacillus casei* YIT 9018 using a microporous glass membrane emulsification system. *Food Engineering and Physical Properties, 68*, 195–200.

Stanton, C., Coakley, M., Murphy, J. J., Fitzgerald, G. F., Devery, R., & Ross, R. P. (2002). Development of dairy-based functional foods. *Science Des Aliments, 22*, 439–447.

Sudoma, A. L. (1990). *Stabilisation of dried bacteria extended in particulate carriers*. United States Patent No. 4, 956, 295. Chr. Hansen's Laboratory, Inc., Milwaukie, Wis.

Teixeira, P., Castro, H., & Kirby, R. (1995a). Spray-drying and a method for preparing concentrated cultures of *Lactobacillus bulgaricus*. *Journal of Applied Microbiology, 78*, 456–462.

Teixeira, P., Castro, H., & Kirby, R. (1996). Evidence of membrane lipid oxidation of spray-dried *Lactobacillus bulgaricus* during storage. *Letters in Applied Microbiology, 22*, 34–38.

Teixeira, P., Castro, H., Malcata, F. X., & Kirby, R. M. (1995b). Survival of *Lactobacillus delbruekii* spp. *bulgaricus* following spray-drying. *Journal of Dairy Science, 78*, 1025–1031.

Teixeira, P., Castro, H., Mohacsi-Farkas, C., & Kirby, R. (1997). Identification of sites of injury in *Lactobacillus bulgaricus* during heat stress. *Journal of Applied Microbiology, 83*, 219–226.

To, B. C. S., & Etzel, M. R. (1997). Spray-drying, freeze-drying, freeze-drying or freezing of three different lactic acid bacteria species. *Journal of Food Science, 63*, 576–585.

Tsvetkov, T., & Brankova, R. (1983). Viability of micrococci and lactobacilli upon freezing and freeze-drying in the presence of different cryoprotectants. *Cryobiology, 20*, 318–323.

Tymczyszyn, E. E., Gomez-Zavaglia, A., & Disalvo, E. A. (2007). Effect of sugars and growth media on the dehydration of *Lactobacillus delbrueckii* ssp. *bulgaricus*. *Journal of Applied Microbiology, 102*, 845–851.

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

Vega, C., & Roos, Y. H. (2006). Invited review: Spray-dried dairy and dairy-like emulsions–compositional considerations. *Journal of Dairy Science, 89*, 383–401.

Walker, D. C., Girgis, H. S., & Klaenhammer, T. R. (1999). The groESL chaperone operon of *Lactobacillus johnsonii*. *Applied and Environmental Microbiology, 65*, 3033–3041.

Wang, Y. C., Yu, R. C., & Chou, C. C. (2004). Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. *International Journal of Food Microbiology, 93*, 209–217.

Wisselink, H. W., Weusthuis, R. A., Eggink, G., Hugenholtz, J., & Grobben, G. J. (2002). Mannitol production by lactic acid bacteria: A review. *International Dairy Journal, 12*, 151–161.

Yoo, B., & Lee, C. M. (1993). Thermoprotective effect of sorbitol on proteins during dehydration. *Journal Agriculture and Food Chemistry, 41*, 190–192.

Zamora, L. M., Carretero, C., & Pares, D. (2006). Comparative survival rates of lactic acid bacteria isolated from blood, following spray-drying and freeze-drying. *Food Science and Technology International, 12*, 77–84.

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