Food Research International 48 (2012) 748–754

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of heat treatment and spray drying on lactobacilli viability and resistance to simulated gastrointestinal digestion

R. Paéz [a], L. Lavari [a], G. Vinderola [b,*], G. Audero [a], A. Cuatrin [a], N. Zaritzky [c], J. Reinheimer [b]

[a] INTA EEA Rafaela, Ruta 34 km 227, Rafaela, Santa Fe, Argentina
[b] Instituto de Lactología Industrial (INLAIN, UNL-CONICET), Facultad de Ingeniería Química, Universidad Nacional del Litoral, 1° de Mayo 3250, Santa Fe, Argentina
[c] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA, UNLP-CONICET) La Plata, Argentina

ARTICLE INFO

Article history:
Received 13 April 2012
Accepted 16 June 2012
Available online 28 June 2012

Keywords:
Heat treatment
Spray drying
Viability
Gastrointestinal digestion
Lactobacilli

ABSTRACT

Commercial probiotic bacteria are delivered mainly as frozen or freeze-dried cultures. However, spray drying is a lower cost technology that could be used for the production of probiotic cultures. In this work we aimed at screening among lactobacilli strains for candidates able to survive to spray drying and to study the effects of a preliminary mild heat treatment and different food matrices on post-drying survival and simulated gastric acid resistance. Heat resistance (survival to exposure at 60 °C for 5 min) in MRS broth or in 10% (wt/vol) skim milk was assessed in 22 strains of *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus acidophilus* and *Lactobacillus plantarum*. Five strains (*L. casei* Nad, *L. plantarum* com, *L. paracasei* A13, *L. plantarum* 8329 and *L. acidophilus* A9) were selected for spray drying in 20% (wt/vol) skim milk and storage at 5, 25 or 37 °C for 75 days. For *L.p.* A13, *L.p.* com and *L.a.* A9 no differences in cell viability were observed due to spray drying. However, for *L.c.* Nad and *L.p.* 8329 cell death due to spray drying was 0.16 and 0.49 log orders CFU ml^{-1} when a mild heat treatment (52 °C for 15 min) was applied and 0.85 and 0.95 log cycles, respectively, without preliminary mild heat treatment, showing that heat treatment enhanced survival to spray drying. The application of a heat treatment was effective for enhancing survival during storage of *L.p.* 8329, irrespective of the storage temperature and period. No significant cell loss at 5 and 25 °C was observed for *L.c.* Nad. For this strain, at 37 °C no cell counts of lactobacilli were observed after 30 days of storage. For *L.a.* A9, *L.p.* com and *L.p.* A13 a reduction in cell viability was observed along storage as temperature increased. Resistance to simulated gastrointestinal digestion was enhanced by spray drying. The application of a mild heat treatment before spray drying may enhance cell survival during storage and the resistance to gastrointestinal digestion. Spray drying might be used for enhancing cell functionality in a strain-dependant way.

1. Introduction

The diversification of the market of probiotic foods relies on the availability of new strains or new formats of probiotic cultures. Until now, fermented dairy products, mainly fermented milks, have been used as the most successful commercial food products for the delivery of probiotic bacteria (Figueroa-González, Quijano, Ramírez, & Cruz-Guerrero, 2011; Saxelin, 2008), being frozen and freeze-dried cultures the commercially available formats of starter and probiotic bacteria. In particular, the production of dried cell cultures is particularly interesting because, unlike frozen cultures, dehydrated cultures demand less storage capacity and lower cost of transport and refrigeration. However, the maintenance of cell viability during drying and storage is a major challenge. Insufficient or too extensive dehydration (moisture >5.0% (wt/wt) or <2.8% (wt/wt), respectively) causes bacterial inactivation (Zayed & Roos, 2004). Presently, industrial manufacture of dried lactobacilli cultures is achieved mainly by freeze-drying, that applies gentle, low-temperature drying conditions. However, freeze-drying is a discontinuous and expensive process with low yields and time and energy demanding (Knorr, 1998; Meng, Stanton, Fitzgerald, Daly, & Ross, 2008). Spray drying is an interesting and promising low-cost alternative because it is relatively inexpensive and allows the continuous production of large amounts of dried cells within short time periods (Gardiner et al., 2000). However, it should be mentioned that cell dehydration may inevitably cause membrane damage and inactivation depending on the technological conditions applied. In spray drying bacterial cultures are exposed to different stresses (osmotic, heat, oxidative) due to the quite harsh conditions of temperature required for product dehydration, which can cause a partial thermal inactivation of cells.

The incorporation of probiotic cultures into fermented dairy products relies almost exclusively on the use of frozen or freeze-dried cultures provided by foreign companies. In particular in Argentina, many medium to big-size dairy industries possess the technological infrastructure for the production of spray dried probiotics. Spray drying can offer a 6 times less expensive alternative every kg of water removed compared

* Corresponding author at: Instituto de Lactología Industrial (INLAIN, UNL-CONICET), Facultad de Ingeniería Química, Universidad Nacional del Litoral, 1° de Mayo 3250, Santa Fe, Argentina. Tel. + 54 342 4530302x4.
E-mail address: gvinde@fiq.unl.edu.ar (G. Vinderola).

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doi:10.1016/j.foodres.2012.06.018

to freeze-drying (Knorr, 1998). However it was observed that the success of its application is highly strain specific (Ananta, Volkert, & Knorr, 2005; Corcoran, Ross, Fitzgerald, & Stanton, 2004; Desmond, Stanton, Fitzgerald, Collins, & Ross, 2002a; Gardiner et al., 2000; Lian, Hsiao, & Chou, 2002; O'Riordan, Andrews, Buckle, & Conway, 2001).

The aim of this work was to screen among lactobacilli strains for candidates able to survive to spray drying and to study the effects of a preliminary heat treatment and different food matrices on post-drying survival and simulated gastric acid resistance.

2. Materials and methods

2.1. Strains and cultures conditions

A total of 22 commercial or collection strains of *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus acidophilus* and *Lactobacillus plantarum* was used in this study (see Table 1). The name of commercial strains was changed in order to avoid any conflict of interest. Strains belong to the culture collections of the INLAIN (UNL-CONICET, Santa Fe, Argentina) and CIDCA (UNLP-CONICET, La Plata, Buenos Aires, Argentina). When needed, fresh overnight (16 h, 37 °C) cultures of cells were obtained in MRS (deMan, Rogosa and Sharp) broth (Biokar, Beauvais, France) after three transfers from frozen (−70 °C) stocks maintained in MRS added with 18% (wt/vol) glycerol (Ciccarelli, Santa Fe, Argentina).

2.2. Heat tolerance assay

Heat resistance was evaluated using the conditions suggested by Simpson, Stanton, Fitzgerald, and Ross (2005) in overnight cultures harvested (6000×*g*, 15 min, 5 °C), washed twice with Phosphate Buffered Saline (PBS) solution (pH 7.1) and resuspended in MRS broth or 10% (wt/vol) skim milk (San Regim, Santa Fe, Argentina). Cell suspensions were placed in a water bath at 60 °C for 5 min and then immediately cooled on an ice bath. Cell counts (MRS agar, 48 h, 37 °C, aerobic incubation) were performed immediately before and after exposure to heat.

Table 1
Heat resistance (cell death in log cycles) of *Lactobacillus* strains after exposure to 60 °C for 5 min in MRS broth or 10% (wt/vol) skim milk.

Species	Strains	Origin	Heat resistance (Δ log CFU ml^{-1} before and after heat treatment)	
			MRS broth	Skim milk
L. casei and *paracasei*	*L.c.* Nad	Commercial product	2.47±0.36	1.38±0.39
	L.p. 27092	Commercial product	2.44±1.14	1.61±0.33
	L.p. 19	Commercial product	3.26±0.70	1.57±0.81
	L.c. A15	Commercial product	2.90±0.13	1.18±0.39
	L.c. A14	Commercial product	3.35±1.07	1.53±0.69
	L.c. Bio	Commercial product	4.15±0.14	2.13±0.04
	L.c. Yak	Commercial product	1.91±0.98	1.34±0.09
	L.p. A13	Commercial product	2.42±0.37	2.20±0.29
L. acidophilus	*L.a.* 1	Commercial product	6.03±0.67	1.49±0.75
	L.a. 5	Commercial product	3.78±0.15	3.00±0.05
	L.a. CSL	Commercial product	5.22±1.17	2.24±0.46
	L.a. A3	Commercial product	3.24±0.70	2.47±0.44
	L.a. A9	Commercial product	2.91±0.59	2.27±1.07
	L.a. 08	Commercial product	1.69±0.48	0.95±0.08
	L.a. 53	Commercial product	2.28±0.86	2.35±0.70
	L.a. CNRZ 1881	CNRZ collection	6.20±0.31	4.91±1.78
L. plantarum	*L.p.* com	Commercial product	2.91±0.20	3.05±0.21
	L.p. 8312	CIDCA collection	3.96±0.96	3.09±0.13
	L.p. 8329	CIDCA collection	3.02±0.29	2.64±1.48
	L.p. 8342	INLAIN collection	3.19±1.03	2.56±1.03
	L.p. 335	Oregon collection	3.18±0.48	2.13±0.94
	L.p. 337	Oregon collection	2.15±0.30	2.07±0.32

2.3. Spray drying in skim milk

L. plantarum com, *L. paracasei* A13, *L. plantarum* 8329, *L. acidophilus* A9 and *L. casei* Nad were selected for spray drying (justified in the Results section). Overnight cultures in MRS broth were harvested (6000×*g*, 15 min, 5 °C), washed twice with PBS solution (pH 7.1), re-suspended in 20% (wt/vol) skim milk and a mild (15 min at 52 °C) heat treatment (MHT) was applied or not (NMHT), according to Desmond, Stanton, Fitzgerald, Collins, and Ross (2001). Cell suspensions were spray dried in a laboratory scale spray dryer (Buchi mini spray dryer model B290, Flawil, Switzerland) by using a constant inlet air temperature of 170 °C, an outlet temperature of 85 °C and a flux of 600 l h^{-1}. Spray drying conditions were those previously suggested as adequate for skim milk (Ananta et al., 2005; Gardiner et al., 2000; Gardiner et al., 2002). Cell suspensions were atomized and sprayed into the drying chamber by using a two-fluid nozzle. The product dried almost instantaneously and the residence time was very low. Three independent replicates were performed for each strain. Spray dried powders were vacuum sealed in individual samples of 10 g. Residual moisture (% wt/wt) was determined in triplicate at 101±1 °C (FIL-IDF 26 A: 1993). Cell counts of lactobacilli were performed before and after spray drying on MRS agar (37 °C, 48 h aerobic incubation) and periodically during the storage at 5, 25 or 37 °C for 75 days.

2.4. Stability of the enhanced resistance to spray drying achieved by the heat treatment

L. casei Nad and *L. plantarum* 8329, for which the mild heat treatment (52 °C, 15 min) enhanced cell survival after spray drying (see Results section) were used. Cell suspensions in 20% skim milk (obtained as described above) were heated at 52 °C for 15 min in water bath and immediately cooled in an ice bath. Cell suspensions were immediately spray dried (as described above) or held at 5 °C for 2 and 4 h before spray drying. Cell counts, before and after spray drying, were performed on MRS agar (48 h, 37 °C, aerobic incubation).

2.5. Spray drying in different matrices and resistance to simulated gastrointestinal digestion

Overnight cultures of *L.c.* Nad, *L.a.* A9 and *L.p.* A13 were suspended in 20% (wt/vol) skim milk, 10% (wt/vol) skim milk + 10% (wt/vol) starch (Glutal S.A., Buenos Aires, Argentina) or 10% (wt/vol) skim milk + 10% (wt/vol) WPC (whey protein concentrate, Arla Foods Ingredients S.A., Argentina) and were spray dried (as described above). Starch and WPC are low cost food ingredients. The use of starch as a carrier was suggested by O'Riordan et al. (2001). No heat mild treatment was applied before spray drying. Cell counts, before and after spray drying, were performed on MRS agar (48 h, 37 °C, aerobic incubation).

For studying the resistance to simulated gastrointestinal digestions, cell suspensions (5 ml) in the different matrices (10% skim milk, 10% skim milk + 10% starch or 10% skim milk + 10% WPC), before or after spray drying, were mixed with the same volume of a simulated 'saliva-gastric' solution containing $CaCl_2$ (0.22 g l^{-1}), NaCl (16.2 g l^{-1}), KCl (2.2 g l^{-1}), $NaHCO_3$ (1.2 g l^{-1}) and 0.3% (w/ v) porcine pepsin (Merck, Darmstadt, Germany), and adjusted to pH 2.50 with 5 M and 1 M HCl (Vinderola et al., 2011). One milliliter samples were removed immediately after mixture (before pH adjustment) and after 30, 60 and 90 min of incubation at 37 °C in a water bath for lactobacilli cell count (MRS, 37 °C, aerobic incubation, 48 h). A sample was removed after 90 min (gastric simulated digestion), centrifuged (6000×*g*, 15 min, 5 °C) and re-suspended in MRS broth containing 0.5% (wt/vol) bovine bile salts (Sigma) at pH 7.4. Cell suspension was incubated at 37 °C for 60 min (bile shock) and counts were performed after incubation (MRS, 37 °C, aerobic incubation, 48 h).

2.6. Statistical analysis

The results of cell counts were transformed to log10 and expressed as mean ± standard deviation or log difference of at least three independent experiments in each trial. In the heat tolerance assay cluster analysis was applied for descriptive method to select the strains for future assays. For the selected strains a nonparametric analysis of variance (Kruskal Wallis test) was performed to detect differences among strains in cell counts after the heat treatment was applied. For the spray drying assay, nonparametric analysis of variance was applied to assess the effect of the heat treatment on the survival during storage. A factorial analysis (heat treatment, storage temperature and time) with repeated measures on the time was applied to study the conservation of powders. In the different matrices tested, an analysis of variance was performed with a factorial arrangement of variables (matrices and strains). Comparisons were performed using the statistical program InfoStat Software (developed by Grupo InfoStat, Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba, Córdoba, Argentina).

3. Results

In order to identify sensitive and tolerant strains of lactobacilli to heat stress, cell suspensions in MRS broth or in 10% (wt/wt) skim milk were exposed to a heat treatment at 60 °C for 5 min. Decays in cell viability can be observed in Table 1. Cell death ranged from 1.91 to 6.20 log orders in MRS broth and from 0.95 to 4.91 log orders in skim milk. The highest decays in cell viability were observed for *L. acidophilus* strains. A cluster analysis was performed to assess the grouping of strains based on the Δ log levels (Δ log CFU ml^{-1} before and after heat treatment) in the two media tested (MRS and skim milk), seeking to form homogeneous groups different from each other (Fig. 1). The dendrogram is a graphical representation to visualize how groups were formed. We used a hierarchical clustering with Euclidean average as a measure of distance. *L.a.* CNRZ 1881 was separated from the rest of the strains, since it presented the biggest loss in cell viability. A second group was composed of strains with erratic behaviors or minimal heat resistance in one of the two media tested (*L.c.* Bio, *L.a.* CSL and *L.a.* 1). The third group of strains was constituted by the remaining strains which presented no significant differences in heat tolerance. Taking into account the lack of significant differences in heat resistance among strains of *L. casei* and *L. paracasei* and their importance in the Argentinian food industry and the probiotic potential of some collection strains of *L. plantarum*, the strains *L. casei* Nad, *L. paracasei* A13, *L. plantarum* com and 8329 and *L. acidophilus* A9 were selected to study the effect of a non-lethal heat treatment on the survival to spray drying and during storage at different temperatures. Milder heat treatment conditions were chosen (52 °C for 15 min) compared to the value chosen in the screening experiment (60 °C for 5 min). This milder heat-treatment did not affect cell viability before spray drying in any case (data not shown). The mean value of moisture of the powders obtained after spray drying was 3.69% ± 0.62 (wt/wt), ranging from 2.02% to 4.89% (wt/wt). Table 2 shows the cell counts of cultures before and after spray drying when a preliminary mild heat treatment was applied or not. No significant differences due to spray drying were observed for *L.p.* A13, *L.a.* A9 or *L.p.* com, with or without mild heat treatment. For *L.c.* Nad and *L.p.* 8329, the mild heat treatment applied was effective in reducing the cell death associated to spray drying, from 0.85 to 0.16 log orders and from 0.95 to 0.49 log orders, respectively (Table 2). Survival of lactobacilli in the vacuum-sealed powders obtained was studied during 75 days of storage at 5 °C, 25 °C and 37 °C (Fig. 2). Generally speaking, a gradual diminution in cell counts was observed along storage as temperature was higher, mainly for *L. plantarum* com (Fig. 2a) and *L. paracasei* A13 (Fig. 2b). By the end of storage period, the highest cell counts were observed in powders kept at 5 °C, for all strains. The mild heat treatment significantly ($p<0.05$) enhanced the survival of *L.p.* 8329 during the storage at the three assessed temperatures (Fig. 2c). For the other strains no significant differences on survival along storage were observed when heat treated and non-heat treated cultures were compared. No significant decay in cell viability was observed for *L.c.* Nad at 5 or 25 °C along storage (Fig. 2e), but at 37 °C no cell counts were observed by day 30 of storage.

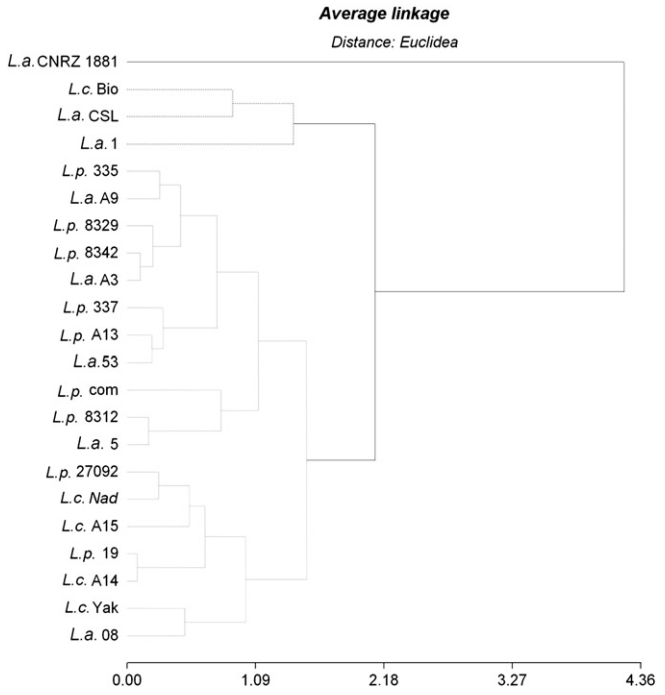

Fig. 1. Average linkage dendrogram for distances between lactobacilli strains clustered in three groups.

For *L.c.* Nad and *L.p.* 8329, a significant loss in cell viability was observed after spray drying when no preliminary mild heat treatment was applied (Table 2). We were interested in determining whether a delay between heat treatment and spray drying might result in the loss of the enhanced capacity to survive spray drying in skim milk. Table 3 shows cell counts obtained in reconstituted spray dried powders when cell suspensions were spray dried 2 or 4 h post heat treatment. No significant differences were observed in any case, showing that the enhanced resistance to spray drying obtained by heat treatment lasted for at least 4 h after its application.

Table 2
Survival of lactobacilli to spray drying (SD) in 20% skim milk with (HT) or without (NHT) previous heat treatment (52 °C for 15 min).

Strain	Heat treatment	Cell counts (log CFU ml^{-1})		Cell death (Δ log before and after SD)
		Before SD	After SD*	
L.c. Nad	MHT	8.63 ± 0.23^{a}	8.46 ± 0.23^{a}	0.16 ± 0.03^{c}
	NMHT	8.73 ± 0.22^{a}	7.88 ± 0.52^{b}	0.85 ± 0.30^{d}
L.p. 8329	MHT	8.93 ± 0.30^{a}	8.44 ± 0.16^{a}	0.49 ± 0.18^{c}
	NMHT	9.01 ± 0.10^{a}	8.06 ± 0.24^{b}	0.95 ± 0.14^{d}
L.p. A13	MHT	8.90 ± 0.11^{a}	8.43 ± 0.50^{a}	0.27 ± 0.23^{c}
	NMHT	8.97 ± 0.03^{a}	8.68 ± 0.20^{a}	0.29 ± 0.21^{c}
L.p. com	MHT	8.91 ± 0.24^{a}	8.55 ± 0.13^{a}	0.37 ± 0.17^{c}
	NMHT	9.12 ± 0.31^{a}	8.68 ± 0.04^{a}	0.45 ± 0.35^{c}
L.a. A9	MHT	9.06 ± 0.07^{a}	8.76 ± 0.13^{a}	0.31 ± 0.05^{c}
	NMHT	9.02 ± 0.00^{a}	8.62 ± 0.16^{a}	0.40 ± 0.16^{c}

[a,b]Cell counts in rows with different superscripted letters are significantly different ($p<0.05$).
[c,d]Δ log in columns for the same strain with different superscripted letters are significantly different ($p<0.1$).
* Spray dried powders were reconstituted to the original liquid volume for the enumeration of viable cells after spray drying (SD).

One strain of each species/group under study was selected for spray drying in different food matrices in order to study the influence of the food matrix (skim milk, starch or WPC) or the technological treatment (spray drying vs. fresh culture) on viability and resistance to simulated gastrointestinal digestion. Skim milk with the addition of a similar amount of starch or WPC was used as protectant. No differences in cell counts were observed before or after spray drying in the different food matrices for any of the strains studied (Table 4). However, when a simulated gastrointestinal digestion was carried out (exposure to hydrochloric acid + pepsin followed by exposure to bovine bile salts), the survival capacity depended on food matrix and on whether cells were digested as fresh or spray dried cultures (Fig. 3). By the end of the simulated gastrointestinal digestion (150 min), cell counts of spray dried *L. paracasei* A13 were ca. 1.5 log orders above of those corresponding to the fresh culture, both in 20% (wt/wt) skim milk. No differences between spray dried vs. fresh culture were observed when the strain was dried or suspended in skim milk + starch. However, lower cell counts were observed for the strain spray dried in skim milk + WPC. By the end of the simulated gastrointestinal digestion (150 min), cell counts of spray dried *L. acidophilus* A9 and *L. casei* Nad were significantly higher than those corresponding to the fresh cultures, irrespective of the food matrix used. The enhanced survival to gastrointestinal digestion due to spray drying ranged from ca. 0.8 to 2 log orders.

4. Discussion

Spray drying, as a technology for producing probiotic cultures, has as advantage its rapidity and relatively low cost, compared to freeze-drying. The technique is highly reproducible and one of the most important issues is that it is available for industrial applications. However, the main problem is the use of high temperature which is

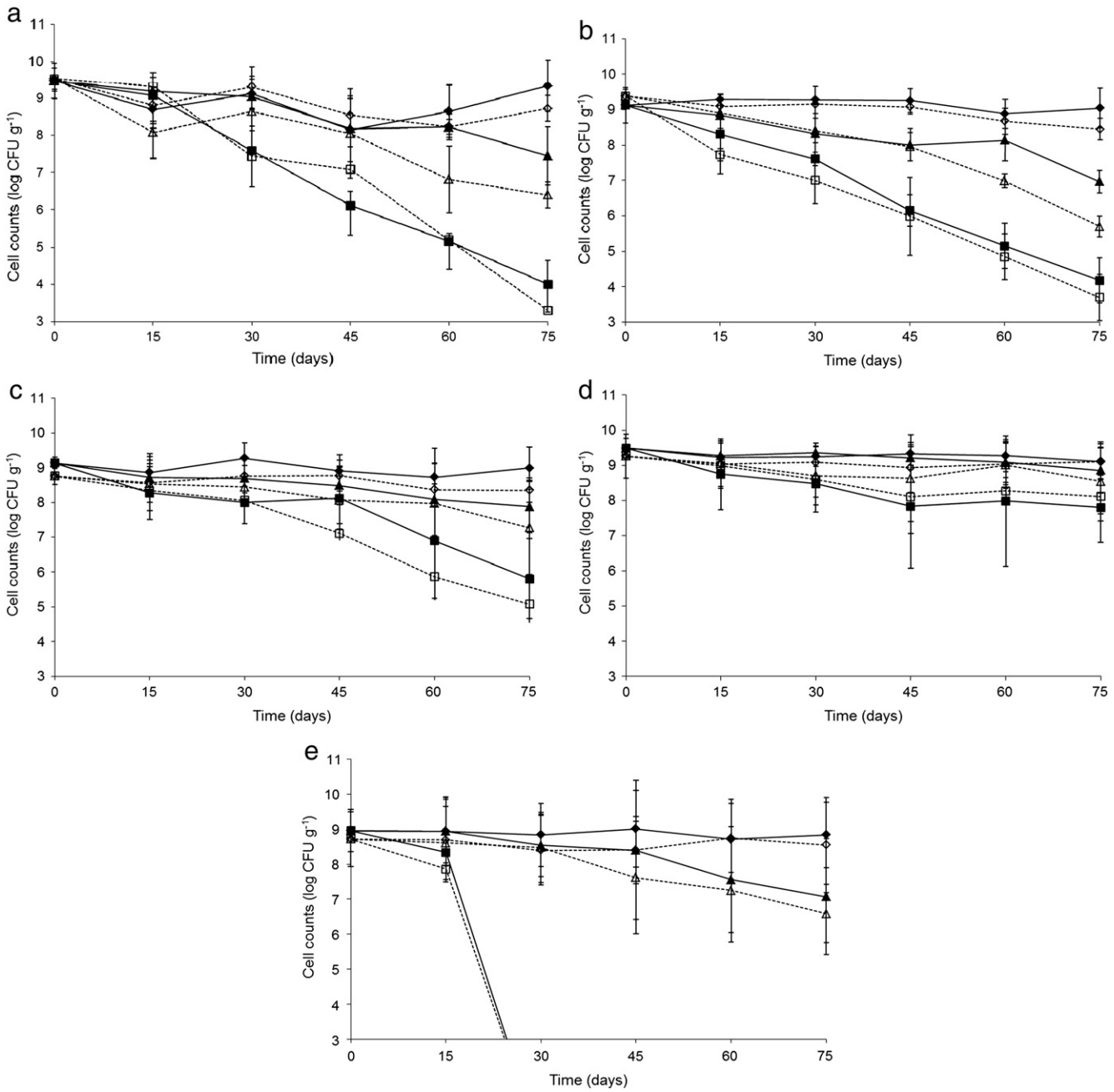

Fig. 2. Survival of spray dried *L.p.* com (a), *L.p.* A13 (b), *L.p.* 8329 (c), *L.a.* A9 (d) and *L.c.* Nad (e), obtained with (solid line) or without (dashed line) previous heat treatment, during the storage at 5 °C (◆), 25 °C (▲) or 37 °C (■).

Table 3
Cell counts of *L. casei* Nad and *L. plantarum* 8329 before or after spray drying (SD). Spray drying was performed after 0, 2 or 4 h of heat treatment (52 °C for 15 min).

Strain	Cell count (log CFU ml^{-1})			
	Before spray drying	After spray drying*		
		After mild heat treatment (h)		
		0	2	4
L.c. Nad	9.23 ± 0.06[a]	9.04 ± 0.08[a]	9.04 ± 0.11[a]	8.82 ± 0.16[a]
L.p. 8329	9.33 ± 0.16[a]	9.10 ± 0.15[a]	8.97 ± 0.26[a]	8.95 ± 0.13[a]

* Spray dried powders were reconstituted to the original liquid volume for the enumeration of viable cells after spray drying.
[a] Cell counts in rows are not significantly different.

not always compatible with the survival of all strains of bacteria (Burgain, Gaiani, Linder, & Scher, 2011). Spray drying has been used for the manufacture of powders from skim-milk based media containing high numbers of viable bacteria from different species (Gardiner et al., 2000; Lian et al., 2002; O'Riordan et al., 2001; Simpson et al., 2005), however the success of its application seems to be, different to freeze-drying, highly strain and environmental condition's dependent. In this study a screening of heat resistance of commercial and collection strains of lactobacilli was performed. Five strains were selected for application (or not) of a mild heat treatment and spray drying and cell viability was studied along storage at three different temperatures. The lasting of the enhanced spray drying resistance was studied too as well as survival to spray drying in different food matrices and during simulated gastrointestinal digestion.

Intrinsic resistance to heat is an important factor for spray drying that will determine its survival to this technological process. Previous studies showed that strains from the same or from related species display different heat resistance (Christiansen, Nielsen, Vogensen, Brogren, & Ardo, 2006) and hence different survival capacity to spray drying (Golowczyc, Silva, Teixeira, de Antoni, & Abraham, 2011; Lian et al., 2002). Gardiner et al. (2000) showed that temperatures lower than 55 °C induced no significant differences in cell resistance to heat. Then a higher temperature (60 °C) was chosen in this study for the screening of cell resistance to heat. Among strains studied, cell resistance was slightly higher in *L. casei* and *L. paracasei* compared to *L. plantarum* and *L. acidophilus*. The majority of *L. casei* and *L. paracasei* strains fell in the bottom of the dendrogram. Additionally, heat resistance was higher when cells were suspended in skim milk compared to MRS broth, due to the heat protective capacity of milk (Corcoran et al., 2004). When *L.a.* CNRZ 1881, *L.c.* Bio, *L.a.* CSL and *L.a.* 1 were excluded from the analysis, no significant differences in heat resistance were detected ($p > 0.05$) among strains.

Five strains of lactobacilli (*L. casei* Nad, *L. paracasei* A13, *L. plantarum* com and 8329 and *L. acidophilus* A9) from the three species/groups studied were chosen on the basis of their importance of present or potential use in the Argentinian food industry, since heat tolerance alone is not an accurate predictor of performance during spray drying (Santivarangkna, Kulozik, & Foerst, 2008). These strains were submitted or not to a preliminary adaptation to mild heat (52 °C, 15 min), as suggested by Desmond et al. (2002a) and then spray dried in 20% (wt/wt) skim milk. For *L. casei* Nad and *L. plantarum* 8329, the preliminary adaptation to heat proved to be effective for enhancing cell resistance to spray drying, as reported by Desmond, Ross, O'Callaghan, Fitzgerald, and Stanton (2002b) for *L. paracasei* NFBC 338. Adverse conditions or stresses during microbial growth can lead to enhanced tolerance responses. Heat adaptation is a well-established phenomenon

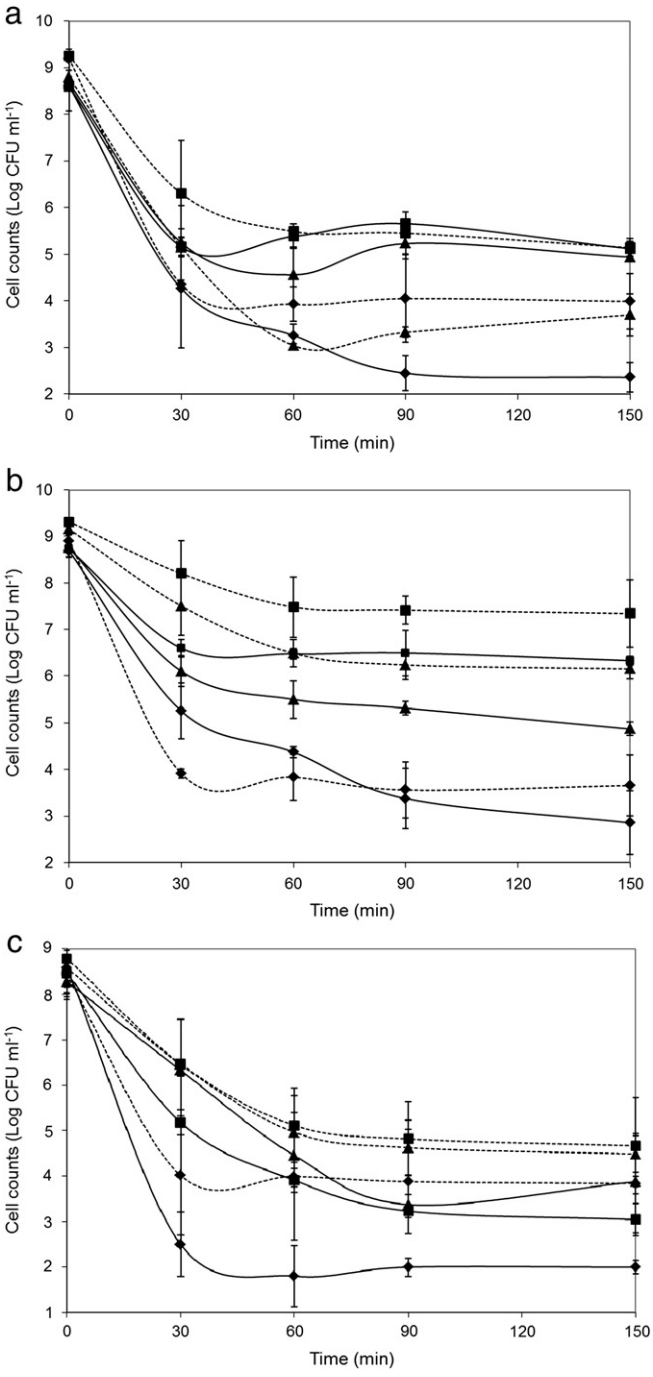

Fig. 3. Survival of *L. paracasei* A13 (a), *L. acidophilus* A9 (b) and *L. casei* Nad (c) as fresh (solid lines) or spray dried cultures (dashed lines) during the simulated gastrointestinal digestion in skim milk (♦), skim milk + starch (■) or skim milk + WPC (▲).

Table 4
Survival of lactobacilli to spray drying (SD) in different dairy matrices.

Strain	Matrix	Cell counts (log CFU ml^{-1})	
		Before SD	After SD*
L. casei Nad	Skim milk	9.01 ± 0.18[a]	8.69 ± 0.07[a]
	Skim milk-starch	9.18 ± 0.25[a]	8.95 ± 0.24[a]
	Skim milk-WPC	9.18 ± 0.14[a]	8.70 ± 0.13[a]
L. acidophilus A9	Skim milk	9.72 ± 0.28[a]	9.32 ± 0.16[a]
	Skim milk-starch	9.68 ± 0.48[a]	9.45 ± 0.31[a]
	Skim milk-WPC	9.68 ± 0.26[a]	9.31 ± 0.32[a]
L. paracasei A13	Skim milk	9.27 ± 0.22[a]	9.12 ± 0.30[a]
	Skim milk-starch	9.34 ± 0.20[a]	9.24 ± 0.27[a]
	Skim milk-WPC	9.44 ± 0.32[a]	9.22 ± 0.53[a]

* Spray dried powders were reconstituted to the original liquid volume for the enumeration of viable cells after spray drying.
[a] Cell counts in row are not significantly different.

described for lactobacilli (De Angelis et al., 2004) and has been proposed as an explanation of why NSLAB can overcome pasteurization and be present during the ripening of certain cheeses. Survival during the storage at 5, 25 and 37 °C was inversely related to the storage temperature, as previously described for lactobacilli (Gardiner et al., 2000). The effect of heat adaptation on a better survival capacity during storage was observed only for *L.p.* 8329. In this case, cell counts were higher at all storage temperatures when cultures were submitted first to the mild heat treatment, confirming that various stresses, such as heat, can make lactic acid bacteria more tolerant to spray drying (Peighambardoust, Tafti, & Hesari, 2011). The application of a non-lethal mild heat stress to enhance survival during spray drying and storage of powders could be potentially used at industrial level. In this case, and due to the larger volumes of cell suspensions to be processed in the industry, a delay in time between the application of the heat treatment and the spray drying could occur. In this regard, we were interested to determine for how long a heat treated cell suspension could be maintained at 5 °C before spray drying, without losing the enhanced resistance to spray drying. This issue was studied for *L.c.* Nad and *L.p.* 8329, the strains that showed an enhanced survival capacity to spray drying when a preliminary mild heat treatment was applied (Table 2). In both cases, the enhanced survival capacity was observed even after 4 h following the heat treatment. This can be explained by a permanent expression of cytosolic heat stress proteins (De Angelis et al., 2004).

Finally, three strains were selected for drying in different food matrices in order to verify the effect of the food matrix, when no heat treatment was applied, on cell resistance to spray drying and during the simulated gastrointestinal digestion. No differences in cell viability were observed due to spray drying in skim milk or skim milk added with an equal proportion of starch or WPC. However, the resistance to simulated digestion was enhanced by spray drying for *L.a.* A9 and *L.c.* Nad in all food matrices assessed and for *L. paracasei* A13 in skim milk. This fact seems to be strain and condition's dependant since O'Riordan et al. (2001) reported that spray dried starch-coated bifidobacteria did not display any enhanced viability compared to free cells when exposed to acid conditions. On the other hand, Fávaro-Trindade and Grosso (2002) showed that microencapsulation of *L. acidophilus* La-05 and *B. lactis* Bb-12 by spray drying in cellulose acetate phthalate enhanced survival at the pH values of the stomach and in bile. It has been lately demonstrated that food matrix in which probiotics are included has a decisive role in determining their viability and functionality during the manufacture of the product and protection of cells upon consumption (Ranadheera, Baines, & Adams, 2010). In line with these findings, it was reported that resistance to acidic conditions in probiotic bacteria might be also conditioned by the food matrix (Saarela, Virkajarvi, Alakomi, Sigvart-Mattila, & Matto, 2006; Vinderola et al., 2011), the pH at which cells are grown (Saarela, Alakomi, Puhakka, & Mättö, 2009; Vinderola et al., 2011), the time at which biomass is harvested (Saarela et al., 2005), the protectant used (Desmond et al., 2002a,b; Saarela et al., 2005; Vinderola et al., 2011) or the storage period (Matto, Alakomi, Vaari, Virkajarvi, & Saarela, 2006; Vinderola et al., 2012). To the best of our knowledge, this is the first report on the capacity of spray drying for modifying the resistance to simulated gastrointestinal digestion in lactobacilli.

5. Conclusions

A non-lethal heat treatment was effective for enhancing the survival of certain strains of lactobacilli to spray drying and during the storage of the powders obtained. The enhanced capacity to overcome spray drying stress was maintained for at least 4 h between heat-stress application and spray drying. The application of spray drying was also effective for enhancing the resistance of the strains to simulated gastrointestinal digestion. Spray drying was effective, in a strain-dependent manner, to produce cultures of probiotic lactobacilli with high survival rate capacity enhanced resistance to simulated gastrointestinal barriers.

Acknowledgments

The authors wish to thank Dr. Graciela de Antoni (CIDCA, UNLP-CONICET, Argentina) for the use of CIDCA collection strains. Luisina Lavari is a doctoral fellow of CONICET (Argentina). This work was funded by the projects PNLEC no. 071021 and AETA no. 281721, supported by INTA.

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