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Resistance of functional *Lactobacillus plantarum* strains against food stress conditions

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ABSTRACT

The survival of three *Lactobacillus plantarum* strains (Lp 790, Lp 813 and Lp 998) with functional properties was studied taking into account their resistance to thermal, osmotic and oxidative stress factors. Stress treatments applied were: 52 °C–15 min (Phosphate Buffer pH 7, thermal shock), H_2O_2 0.1% (p/v) – 30 min (oxidative shock) and NaCl aqueous solution at 17, 25 and 30% (p/v) (room temperature – 1 h, osmotic shock). The osmotic stress was also evaluated on cell growth in MRS broth added of 2, 4, 6, 8 and 10% (p/v) of NaCl, during 20 h at 30 °C. The cell thermal adaptation was performed in MRS broth, selecting 45 °C for 30 min as final conditions for all strains. Two strains (Lp 813 and Lp 998) showed, in general, similar behaviour against the three stress factors, being clearly more resistant than Lp 790. An evident difference in growth kinetics in presence of NaCl was observed between Lp 998 and Lp 813, Lp998 showing a higher optical density (OD_{570nm}) than Lp 813 at the end of the assay. Selected thermal adaptation improved by 2 log orders the thermal resistance of both strains, but cell growth in presence of NaCl was enhanced only in Lp 813. Oxidative resistance was not affected with this thermal pretreatment. These results demonstrate the relevance of cell technological resistance when selecting presumptive "probiotic" cultures, since different stress factors might considerably affect viability or/and performance of the strains. The incidence of stress conditions on functional properties of the strains used in this work are currently under research in our group.

1. Introduction

Lactobacillus plantarum is a versatile species of lactic acid bacteria (LAB), which can be found in different ecological niches and shows fermentative ability on milk, vegetables, coffee, meat and silage. This species was also repeatedly found in the gastrointestinal tract of humans and animals (Chibanni-Chennoufi et al., 2004). Some *L. plantarum* strains are considered "potential probiotics". FAO/WHO (2002) defined the probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". This definition, though claimed 12 years ago, remains valid. *Lactobacillus* and *Bifidobacterium* genera are the most commonly used as potential probiotics and they are included in functional fermented food (Salminen and Gueimonde, 2004; Soccol et al., 2010). In particular, *L. plantarum* strains emerge as potential probiotics able to also act as starter, an advantage over most probiotic species currently used.

The use of probiotic strains in the functional food industry requires some technological considerations. To exert a benefit effect on the human host, probiotics must reach viable in the intestine in a high number (at least 10^6 UFC/ml). To achieve this purpose, strains must be selected by taking into account their survival natural capacity during the production steps, storage and distribution of functional food, as well as their resistance to passage through the host's gastrointestinal tract. Thus, probiotic selection must consider not only evaluation of their functional properties *in vitro* and *in vivo* but also their technological ones, framing the microorganism within the industrial reality (Makinen et al., 2012).

LAB used in fermented food processes are exposed to several adverse conditions (stress factors), even during culture preparation and storage like in the manufacture technological process. Stress factors involved depend on the conservation method and the manufactured food characteristics. In fact, low pH, lyophilization, dried spray, freezing, temperature, osmotic factor and oxidative compounds presence could significantly affect the viability and performance of strains when used in a productive process (Zotta et al., 2008). As other bacteria, LAB have developed sophisticated

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defence mechanisms against stress factors, which have allowed the evolution and survival of microorganisms along the time (van de Guchte et al., 2002; Serrazanetti et al., 2009). In particular, it is reported that *L. plantarum* species maintains viability after their passage through the gastrointestinal tract (GIT) in humans and other mammals, and some strains could provide diverse therapeutic properties to the host (Zago et al., 2011).

The aim of this work was to evaluate the intrinsic resistance of three *L. plantarum* strains, postulated previously as potential probiotics, against heat-, osmotic- and oxidative stress, factors commonly present in fermented food manufacture or microbial conservation process (spray drying as example). The possibility to improve their resistance was also studied, considering the potential adaptation mechanism developed by these strains to temperature.

2. Material and methods

2.1. Strains and culture conditions

L. plantarum strains used in this work (Lp 998, Lp 813 and Lp 790) were isolated from Italian and Argentinean cheese and were proposed as potential probiotic microorganisms in a previous study (Zago et al., 2011). The strains were stored frozen at −20 °C and −80 °C, in MRS broth (Biokar, Beauvois, France), added of 15% (v/v) of glycerol as cryoprotective agent. They were routinely reactivated in MRS broth (24 h−34 °C) and stocked in fridge.

2.2. Growth kinetics

Over night cultures (MRS broth, 18 h−34 °C) were inoculated (2%, v/v) in MRS broth and incubated 20 h at 34 °C. Absorbance ($D.O._{570\ nm}$) values were determined at intervals of 30 min using a Multiskan FC Microplate Photometer (Thermo Fisher Scientific Inc.). Values were plotted and it was determined, for each strain, the incubation time needed to reach the same cell physiology stage (stationary phase growth). The μ_{max} (maximum specific speed, $\mu_{max} = \ln OD_f - \ln OD_0/\theta_f - \theta_0$; OD_f = final optical density; OD_0 = initial optical density; θ_f = final time; θ_0 = initial time) were calculated for each growth kinetics. Assays were performed by triplicate in independent trials.

2.3. Thermal stress

Strains cultures (MRS broth, 34 °C) in stationary phase growth (14 h−18 h, depending on the strain) were centrifuged (6000 *g*−10 min, 15 °C), washed twice with 10 mM buffer phosphate solution pH 7 (PB7) and suspended in the same buffer (Zotta et al., 2008; Parente et al., 2010). The suspensions were heated 35 min at 52 °C and samples were taken at predetermined time intervals (5, 10, 15, 25 and 35 min). After the thermal treatment, samples were immediately cooled and bacterial counts of viable cells were performed in MRS agar (34 °C−72 h). Cultures suspensions maintained for 35 min at room temperature were used as controls. Resistance index (RI), defined as $RI = \log N_0/N_f$ (N_0 = initial cell count; N_f = final cell count), was calculated in each case. Kinetics was plotted and the mathematical function values determined using the Origin Pro 8 software (Origin Lab Corporation). Assays were performed by triplicate in independent trials.

2.4. Oxidative stress

As previously detailed, strain cultures in stationary phase growth were centrifuged, washed twice with PB7 and suspended in the same volume of 0.1% (p/v) hydrogen peroxide (H_2O_2) solution and maintained at room temperature for 30 min (Parente et al., 2010). At predetermined time intervals (10, 20 and 30 min), samples were taken and viable cells counts were performed in MRS agar (34 °C−72 h). Cells suspended in distilled water and subjected to the same conditions of time and temperature were used as controls. In all cases, RIs were calculated, kinetics plotted and the mathematical function values determined using the Origin Pro 8 software (Origin Lab Corporation). Assays were performed by triplicate in independent trials.

2.5. Osmotic stress

Two methodologies were used as follows: (i) cells in stationary phase growth were washed twice with PB7, then suspended in NaCl aqueous solutions at diverse concentrations (17, 25 and 30% w/v) and lastly maintained at room temperature during 1 h (Parente et al., 2010). Cells suspended in distilled water and maintained in the same conditions of time and temperature were used as controls. Cell counts (MRS agar, 72 h at 34 °C, in microaerophilia) were performed before and after incubation time and the corresponding RIs calculated; ii) growth kinetics carried out at diverse NaCl concentrations (De Angelis et al., 2004). For this, cells in stationary phase growth were washed twice with PB7 and then suspended in MRS broth. These suspensions were used to inoculate (2%, v/v) MRS broth added of diverse concentrations of NaCl (2, 4, 6, 8 and 10%, p/v). MRS without salt was used as bacterial growth control. Absorbance ($D.O._{570\ nm}$) values were taken at intervals of 30 min (as item *2.2*) during 20 h at 34 °C. The growth kinetics was plotted and the μ_{max} calculated. Assays were performed by triplicate in independent trials.

2.6. Thermal pre-treatment (adaptation) and stress

Thermal pre-treatment was performed in MRS broth, at temperature of 45 °C (10 °C over the optimal growth temperature, approx.) (De Angelis and Gobbetti, 2004). Cells in stationary phase growth, washed twice with PB7 and suspended in fresh MRS broth, were incubated in bath for 45 min at 45 °C, taking samples at predetermined time intervals (15, 30 and 45 min). Then, cells were exposed to a thermal stress of 55 °C for 15 min, selected on the basis of the destruction kinetics obtained previously (see Results). RIs were calculated for each adaptation time after thermal treatment. The thermal adaptation effect on oxidative stress was studied by applying a thermal pre-treatment of cells in MRS broth, at 45 °C, for 30 min. This time−temperature combination was selected based on the experience explained in the previous paragraph (see Results). After that, cells were washed twice with PB7, suspended in 0.1% (p/v) hydrogen peroxide (H_2O_2) solution and maintained at room temperature for 30 min. The corresponding RIs values were calculated.

The influence of thermal adaptation on growth kinetics in MRS broth added of NaCl was also studied. The aforementioned thermal pre-treatment (45 °C, 30 min) was applied and the cells washed and suspended in fresh MRS broth were used to inoculate (2% v/v) MRS broth with and without NaCl (4 and 6% p/v). Growth kinetics was carried out as previously described and the μ_{max} calculated. All assays were performed by triplicate in independent trials.

2.7. Statistical treatment

Data processing was made using one-way ANOVA and Student test (*t*), using the IBM SPSS® Statistics Version 2.0.

3. Results

3.1. Growth kinetics

The growth kinetics obtained for the three (3) strains are shown in Fig. 1. Clearly, Lp 790 presented a slower growth rate than Lp 813 and Lp 998. In fact, the μ_{max} for Lp 790 was 0.501 Δln OD/h, lower than Lp 813 and Lp 998, which values were 0.677 and 0.657 Δln OD/h, respectively (Fig. 1). The time required for each strain to reach the same cell physiology stage was determined in this study. This fact assured that comparison points among strains were located at the same stage of cell growth. According to these results, the incubation time selected was 14 h for Lp 998 and Lp 813 and 18 h for Lp 790 (indicated in Fig. 1).

3.2. Thermal stress

The results obtained are shown in Fig. 2. Lp 790 was more sensitive against temperature factor than Lp 813 and Lp 998 throughout the experience. Moreover, Lp 813 and Lp 998 showed similar surviving levels. On the basis of these results, 15 min was selected as the thermal shock time to be applied in further experiences. In fact, at this point the cell count reductions were enough to detect a potential improvement of the thermal resistance after the thermal adaptation. The RI mean values corresponding to the thermal shock applied (15 min, 52 °C, PB7) were of 5.30 for Lp 790, 3.30 for Lp 998 and 3.60 for Lp 813. Data fitted with a second order polynomial in all cases and the correspondent coefficients are presented in Table 1.

3.3. Oxidative stress

Results obtained are shown in Fig. 3. Strain behaviour was similar to that obtained in the thermal treatment. Lp 790 resulted more sensitive than Lp 813 and Lp 998 and similar values between these last two strains were found. In this case, and on the basis of the reasons previously explained (Item 3.2), an exposure of 30 min was selected for further studies. After this selected oxidative shock (30 min, 0.1% p/v H_2O_2, room temperature), the RI mean values were 5.45 for Lp 790 and 2.85 for Lp 998 and Lp 813. Data fitted with a second order polynomial in all cases and the correspondent coefficients are presented in Table 2.

3.4. Osmotic stress

All the strains showed high osmotic pressure resistance against NaCl concentrations used (Table 2), with RI mean values lower than 0.3. Lp 813 was slightly affected at a concentration of 30% (p/v), with a RI = 0.54. This result showed a significant difference (one way ANOVA test, $\alpha = 0.05$) against those obtained for Lp 790 and Lp 998.

Regarding growth kinetics in MRS broth added of NaCl, 2% (p/v), the growth rate of strains was not affected, since none of them was able to grow at a concentration of 8% (p/v) (data not shown). NaCl 4% (Fig. 4A) and 6% (Fig. 4B) in culture media produced a delayed growth rate for all the strains. Lp 813 would appear to be the more affected by osmotic stress (by NaCl). This fact could be confirmed by calculating the μ_{max} values, being 0.338 and 0.191 Δln OD/h for Lp 813 (4% and 6% respectively) against 0.354 and 0.228 Δln OD/h for Lp 790 and 0.524 and 0.337 Δln OD/h for Lp 998 (Fig. 4A and B). Clearly, Lp 998 showed the highest intrinsic resistance to osmotic stress.

3.5. Thermal pre-treatment (adaptation) and stress

This assay was performed only on Lp 998 and Lp 813 strains, the most resistant against the three stress factors studied. Optimal thermal adaptation treatment for each strain was studied by analysing the cell survival after the exposure to the previous selected thermal shock (55 °C–15 min). These results are shown in Fig. 5, where the effect of thermal adaptation (45 °C) at different incubation times and following thermal shock was plotted. The RI mean values after the thermal stress were similar for both strains, either for 30 and 45 min. In fact, Lp 813 and Lp 998 strains showed mean values of 0.91–0.60 and 0.94–0.62 for 30 and 45 min of thermal adaptation, respectively. No significant differences ($\alpha = 0.05$) between RI values (30 and 45 min) of all the strains were demonstrated by the statistical treatment (*t* Student test). Thermal

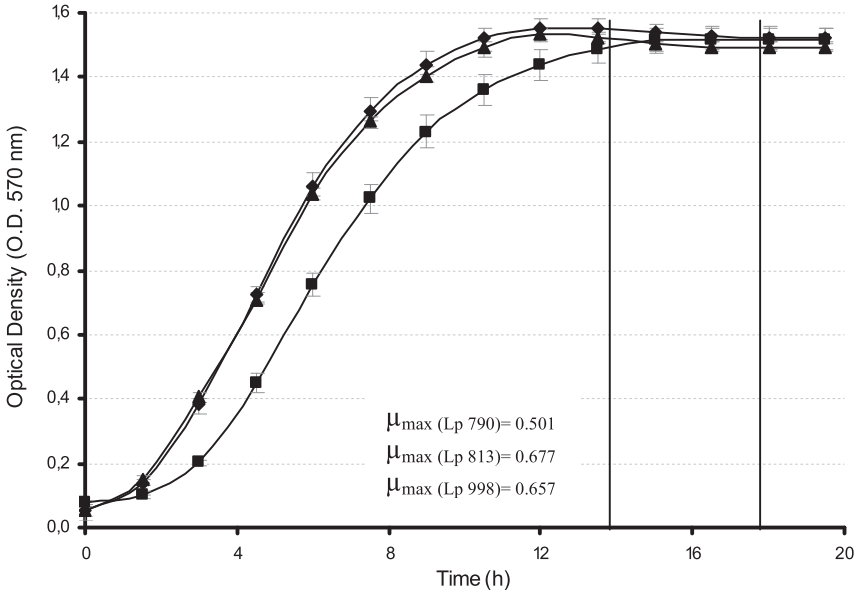

Fig. 1. Growth kinetics at 30 °C (20 h) of Lp 813 (▲), Lp 998 (◆) and Lp 790 (■). Lines at 14 h and 18 h indicate the selected incubation times of each strain to reach the same physiological state. The μ_{max} (ln OD_f – ln $OD_0/\theta_f - \theta_0$, OD_f = final optical density; OD_0 = initial optical density; θ_f = final time; θ_0 = initial time) values are shown in curves.

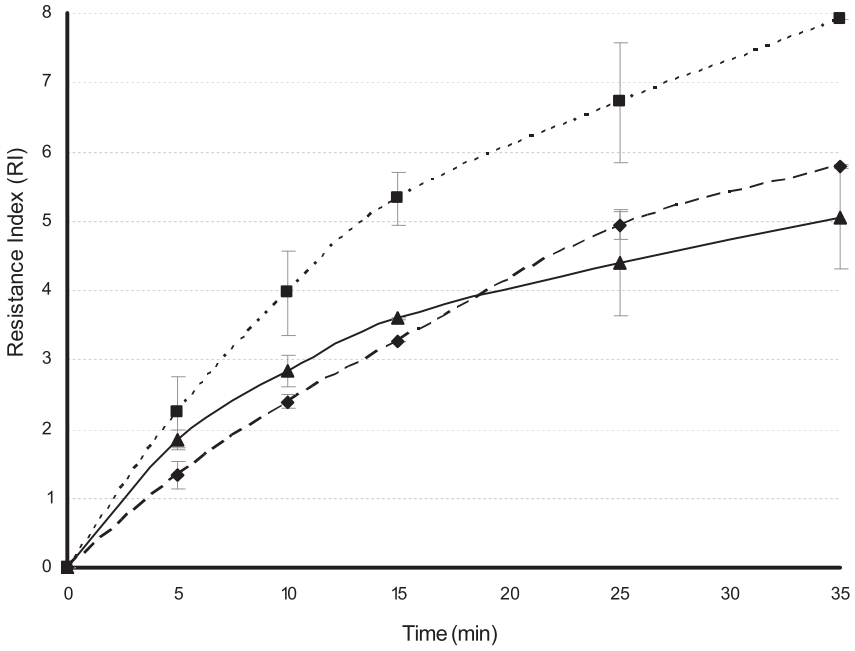

Fig. 2. Thermal resistance index (RI = log N_0/N_f, N_0 = initial cell count; N_f = final cell count) of Lp 813 (▲), Lp 998 (◆) and Lp 790 (■) subjected to 52 °C in phosphate buffer (10 mM, pH 7) at diverse times.

Table 1
Mathematical function data (second order polynomial) of thermal and oxidative destruction of *Lactobacillus plantarum* strains in stationary growth stage.

Strain	Thermal stress (55 °C)[a]			Oxidative stress (0.1% p/v H_2O_2)[b]		
	Quadratic coefficient (a)	Lineal coefficient (b)	Regression coefficient (R^2)	Quadratic coefficient (a)	Lineal coefficient (b)	Regression coefficient (R^2)
Lp 790	−0.006	0.442	0.994	0.007	−0.034	0.977
Lp 998	−0.003	0.268	0.999	0.005	−0.063	0.991
Lp 813	−0.005	0.318	0.979	0.005	−0.052	0.982

[a] Treatment performed for 35 min.
[b] Treatment performed for 30 min.

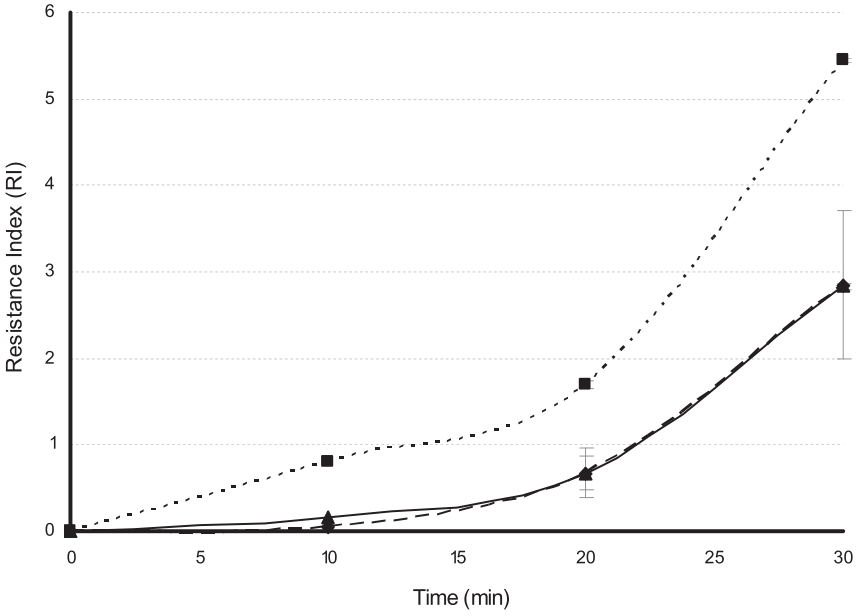

Fig. 3. Oxidative resistance index (RI = log N_0/N_f, N_0 = initial cell count; N_f = final cell count) of Lp 813 (▲), Lp 998 (◆) and Lp 790 (■) subjected to oxidative shock (H_2O_2 0.1% w/v) at diverse times.

Table 2
Resistance Index (RI) values of *Lactobacillus plantarum* strains in stationary growth stage, when maintained for 1 h a different NaCl concentration.

Strain	Resistance Index (RI) values[a] (1 h in NaCl solution at w/v)		
	17%	25%	30%
Lp 790	0.17 ± 0.01[b]	0.10 ± 0.09[b]	0.10 ± 0.11[b]
Lp 998	0.32 ± 0.10[b]	0.19 ± 0.10[b]	0.54 ± 0.08[c]
Lp 813	0.06 ± 0.05[b]	0.23 ± 0.30[b]	0.17 ± 0.11[b]

[b, c]Different letters in the same column indicate significant differences by using One-Way ANOVA ($\alpha \leq 0.05$) test.
[a] RI = log N_0/N_f (N_0 = initial cell count; N_f = final cell count) expressed as mean ± standard deviation.

adaptation (30 min) clearly improved cell resistance against thermal shock. In this case, IR mean values decreased from 3.31 to 0.94 for Lp 998 and from 3.04 to 0.91 for Lp 813.

The response of the strains against the oxidative shock (0.1% w/v H_2O_2 − 30 min), prior thermal adaptation (45 °C−30 min) is shown in Fig. 6. No significant differences (*t* Student test, $\alpha = 0.05$) were found in the strains before and after thermal adaptation. The RI mean values were 2.85 and 1.71 for Lp 998 and 2.83 and 2.60 for Lp 813.

Thermal adaptation remarkably affected the growth of Lp 813 in presence of NaCl. The growth rate improvement at 4% and 6% of NaCl is shown in Fig. 7A. When adaptation was assayed for this strain, the final optical density (4% NaCl) reached the same value (19 h, $A_{570\ nm}$−1.50) as the strain in MRS without salt, but 6 h later. At 6% NaCl, an improvement in osmotic resistance was also observed, until final values (19 h) of $A_{570\ nm}$−1.16. The μ_{max} values of the curves improved from 0.364 to 0.430 Δln OD/h and from 0.191 to 0.210 Δln OD/h, by 4% and 6% of NaCl, respectively. On the contrary, thermal adaptation did not improve Lp 998 growth in MRS broth added of NaCl (Fig. 7B). In fact, the μ_{max} values were 0.514 and 0.488 Δln OD/h (4% NaCl) and 0.337 and 0.240 Δln OD/h (6% NaCl), non-adapted and adapted strain respectively. Even if the μ_{max} value obtained for adapted cells of Lp 998 developed at 6% NaCl was notably lower than the value obtained for not adapted cells, this point could be explained by the large variability in values obtained in the latter case. Lastly, it is interesting to remark that, although Lp 998 was intrinsically more resistant to osmotic stress than Lp 813, similar growth kinetics were obtained for both strains when thermal adaptation was applied.

4. Discussion

Lactobacillus genus is widely used as starter to manufacture a broad variety of fermented products, as cheese, yoghurt, vegetables, etc. The extended use of *Lactobacillus* species is mainly related to their GRAS (Generally Recognized as Safe) status, nevertheless several strains were also shown to have health improving effects (De Angelis and Gobbetti, 2004; Soccol et al., 2010). To produce some positive effects on health, a high count (>10^6 CFU/g) of viable cells must get to the intestinal region (Gobbetti et al., 2010). Survival and technological performance of strains used as starters and/or adjuncts after subjecting them to adverse conditions (such as temperature, pH, lack of nutrients, high osmotic pressure, etc.), either during storage or fermentation, are essential for a successful application (van de Guchte et al., 2002; Serrazanetti et al., 2009). In this sense, the “robustness” of the strains against stress factors as selection criteria is crucial for a correct choice.

The ability to tolerate and adapt to several stress conditions is one of the main forces of the bacterial evolution (van de Guchte et al., 2002; Serrazanetti et al., 2009; De Angelis and Gobbetti, 2011), allowing cells to survive in unfavourable environments. In this study, three potential probiotic strains of *L. plantarum* were tested considering their intrinsic resistance to heat-, oxidative- and osmotic stress. Clearly, two of them (Lp 813 and Lp 998) were more resistant than the third one (Lp 790), in the same cell physiology state, and against heat and oxidative stress conditions applied. Regarding osmotic stress, the three strains showed the same resistance. The higher tolerance of stationary phase cells against physical stress factors compared with cells in exponential phase is a fact widely reported in literature for *L. plantarum* (De Angelis et al., 2004; Parente et al., 2010) and other LAB (van de Guchte et al., 2002). The adaptive response of cells during growth involves the activation of some genes to cope with diverse stress conditions (exhaustion of nutrients, acidity, heat, etc.) and maintain viability (De Angelis and Gobbetti, 2011). This cell adaptation leads to expressing a general response to stress obtaining more resistant cells, which could survive to adverse growth conditions. It is necessary to remark that, in this study, culture conditions were not controlled regarding pH factor (free pH). As acknowledged, pH decreases during LAB culture growth and General Stress Proteins, such as DnaK, DnaJ, GroES, GroEL chaperons, are synthesized. These proteins are also involved in heat and osmotic resistance, thus it is expected that these cells could also be more resistant against those stress factors than cells obtained using controlled pH (~6.8) (Carvalho et al., 2004).

Heat shock (55 °C−15 °C, MRS broth) applied in our study allowed us to successfully discriminate among strains, yielding viability reductions by 3 logarithmic orders (approx.) for Lp 998 and Lp 813 and 5 log orders for Lp 790. This thermal treatment was also tested on Lp 8329 (CIDCA Collection, Buenos Aires, Argentina) as control, obtaining for this strain a viability reduction similar to that of Lp 998 and Lp 813 (data not shown). The heat resistance of Lp 8329 had been previously compared with other potential probiotic strains (belonging to *L. plantarum*, *Lactibacillus casei*, *Lactobacillus paracasei* and *Lactobacillus acidophilus* species), showing that this strain had a relatively high resistance against this stress factor (temperature) (Paez et al., 2012). Consequently, it would be possible to think that Lp 998 and Lp 813 also present a relative high resistance against thermal stress. Even though there were many studies about cell stress resistance of LAB, in general, most of them were made on a limited number of strains. Moreover, only a few studies reported the resistance of LAB against diverse stress factors. Zotta et al. (2008) screened 56 strains of *Streptococcus thermophilus*, *Streptococcus salivarius* and *Streptococcus macedonicus* and related dairy streptococci for their tolerance against acid-, osmotic-, oxidative- and heat stress. Although *S. thermophilus* is a species confined to dairy environments, a remarkably large diversity in stress tolerance was found. For the 37.5% of the strains, the authors also demonstrated a higher heat resistance of cells in stationary phase than in exponential phase. Parente et al. (2010) reported the screening of sixty-three (63) strains of the taxonomically related species *L. plantarum* subsp. *plantarum*, *L. plantarum* subsp. *argentoratensis*, *Lactobacillus paraplantarum* and *Lactobacillus pentosus* regarding diverse stress conditions (acid-, alkaline-, heat-, oxidative-, osmotic-, detergent- and starvation factors). The authors used the same thermal treatment (55 °C−15 min) selected by us, although the tested cells were harvested in exponential growth state. Viability reductions ranging from 4 to 7 log orders for most strains tested were demonstrated.

Dehydration of bacterial cells results into serious oxidative stress and a consequent cell membrane damage due to the formation of reactive oxygen species (ROS), which in turn cause lipid peroxidation and deesterification, proteins denaturation and damage in cell nucleic acids. In a metabolically active cell state, these ROS are trapped by the antioxidant defence system in a

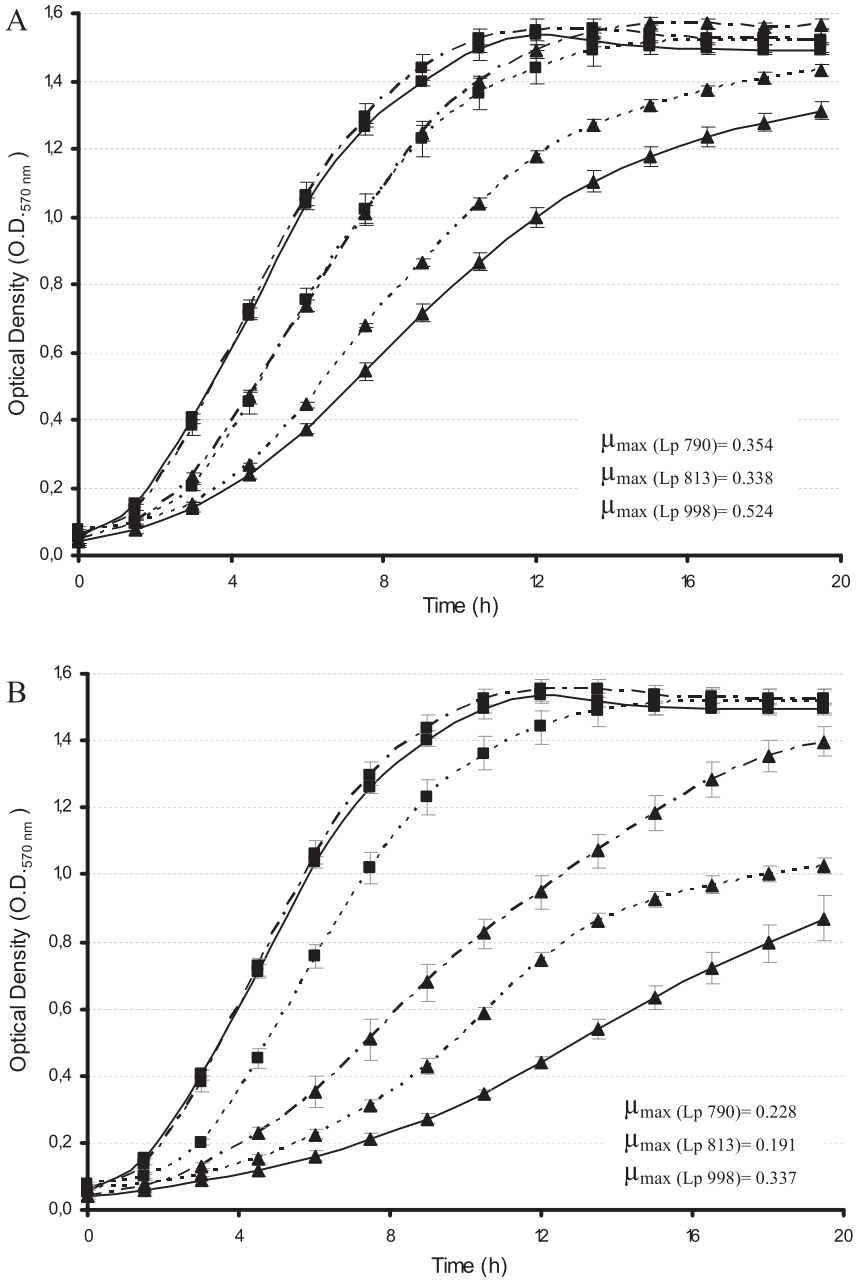

Fig. 4. Growth kinetics at 30 °C (20 h) of Lp 813 (full line), Lp 998 (dash-drop line) and Lp 790 (dash line) in MRS broth with (■) and without (▲) NaCl 4% (A) and 6% (B). The μ_{max} ($\ln OD_f - \ln OD_0/\theta_f - \theta_0$, OD_f = final optical density; OD_0 = initial optical density; θ_f = final time; θ_0 = initial time) values are shown in curves.

normal way, but during prolonged dry storage the cell membranes are more susceptible to ROS attack (Nag and Das, 2013). Conversely, Rodrigues et al. (2011) reported that the presence of oxygen during storage after spray process did not affect the cell viability of probiotic strains *Lactobacilus acidophilus* Ki, *L. paracasei* L26 and *Bifidobacterium animalis* BB-12. Anyway, considering the potential incidence of this factor on cell viability post spray dried, the study of strain resistance is essential if this storage method would be used. In our study, H_2O_2 was used as an oxidative agent to evaluate this stress factor. As for heat stress, Lp 998 and Lp 813 were more resistant than Lp 790. Although *L. plantarum* species is relatively resistant to oxidative stress, a large variability of H_2O_2 tolerance has been previously reported for stationary phase cells (Parente et al., 2010). Interestingly, these authors found that strains most tolerant to heat were also the most tolerant to oxidative stress. These results were similar to those found in our study. The same behaviour was also demonstrated by diverse bifidobacteria species (Simpson et al., 2005). Intraspecies diversity of *Lactobacillus sakei* response to various oxidative compounds, even H_2O_2, was studied by Guilbaud et al. (2012). Wide phenotypic response diversity was found for all stress compounds used, mainly for the disulfide stress generator. In general, it is well accepted that the intrinsic resistance against oxidative compounds leads to an improved viability post-spray dried.

Osmotic stress is other stress factor involved in the spray dried process. Dehydration and heat stress are the main cause of critical

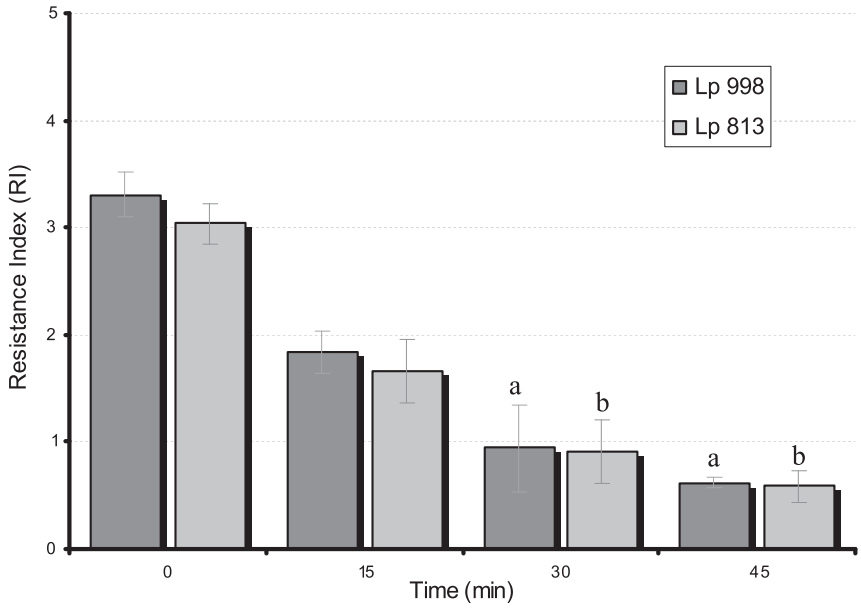

Fig. 5. Thermal resistance index (RI = log N_0/N_f, N_0 = initial cell count; N_f = final cell count) of Lp 813 and Lp 998 without and with thermal adaptation (45 °C) at diverse times. Same letters indicate not significant difference ($\alpha = 0.05$) using Student test (t).

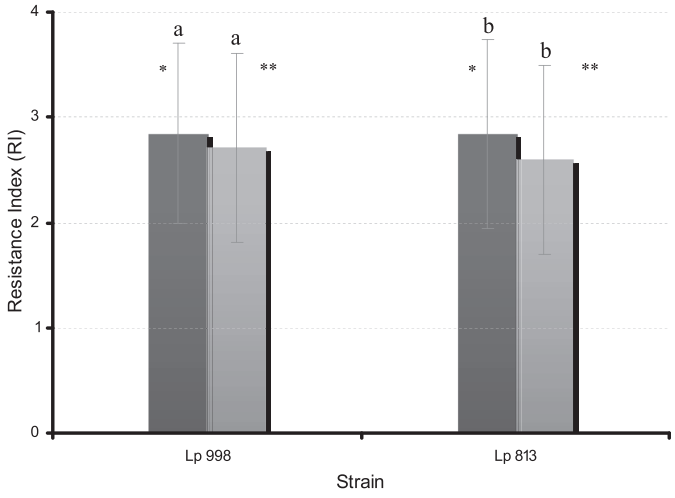

Fig. 6. Oxidative resistance index (RI = log N_0/N_f, N_0 = initial cell count; N_f = final cell count) of Lp 813 and Lp 998 without and with thermal adaptation (45 °C–30 min), subjected to oxidative shock (H_2O_2 0.1% w/v – 30 min). Same letters indicate not significant difference ($\alpha = 0.05$) using Student test (t).

cell damage and diminished cell viability (Salar-Behzadi et al., 2013). In particular, it is well known the osmotic resistance of *L. plantarum* and, due to this attribute, its ability to ferment food with high salt concentration (0.5–10 %) (Chamkha et al., 2008; Milesi et al., 2008; Gómez-Ruiz et al., 2008). In our work, the three strains resisted up to 30% (w/v) NaCl (close to saturation concentration at room temperature). Desmond et al. (2004) reported a reduction by 1 log order for *Lactococcus lactis* NZ9800 and *L. paracasei* NFBC 338 strains (29% w/v NaCl – 1 h), tested in GM17 and MRS media respectively. Most *S. thermophilus* strains (76.8%) studied by Zotta et al. (2008) showed a high tolerance to the treatment assayed (12% w/v NaCl – 30 min), while Parente et al. (2010) reported that most (60 of a total of 63) *L. plantarum* group strains maintained unalterable their viability against 17.5% (w/v) NaCl for 1 h.

In addition, we have also tested the influence of NaCl concentration on the strain growth kinetics. This assay allowed us to observe differences among the respective curves. In fact, a low growth in presence of salt (4 and 6%) was shown by Lp 813. As known, the bacterial response to hyperosmolarity is related to the ability of cells to accumulate osmoprotective compounds (Pichereau et al., 2000). Osmotic stress can lead to accumulation of humectant molecules (for example, sugar) or synthesis of osmoregulatory compounds to maintain osmotic balance. Compatible solutes may play a role in osmoprotection; a protective effect during drying has already been reported for such compounds as betaine, carnitine, and mannitol. The mechanism behind this effect remains to be fully elucidated, but increased levels of compatible solutes play positive roles in cell survival and enzyme activity (Carvalho et al., 2003).

The ability of the *Lactobacillus* species to adapt to different environmental conditions is variable. In particular, *L. plantarum* is an extremely versatile species, commonly isolated from fermented food and found as a natural inhabitant of the human gastrointestinal tract (De Angelis and Gobbetti, 2011). One of the most common consequences of a given stress condition in bacteria is the induction of protein misfolding, leading to partial or total metabolic block. When this situation occurs, a set of molecular effectors, generally related to chaperones, assist in proper protein folding and in the degradation of misfolded proteins, orchestrated around a tightly-regulated network. Heat adaptation, such as other stress factors, involves the synthesis of these protein chaperons (HSPs) in all lactobacilli species reported (Serrazanetti et al., 2009, Sánchez et al., 2012). In our study, the thermal adapted strains improved their thermal resistance by approx. 2 log orders. The cross stress resistance was found only for Lp 813 but not for Lp 998. Studies focused on the characterization of the induced proteins for each strain are being currently performed in order to explain their dissimilar behaviour.

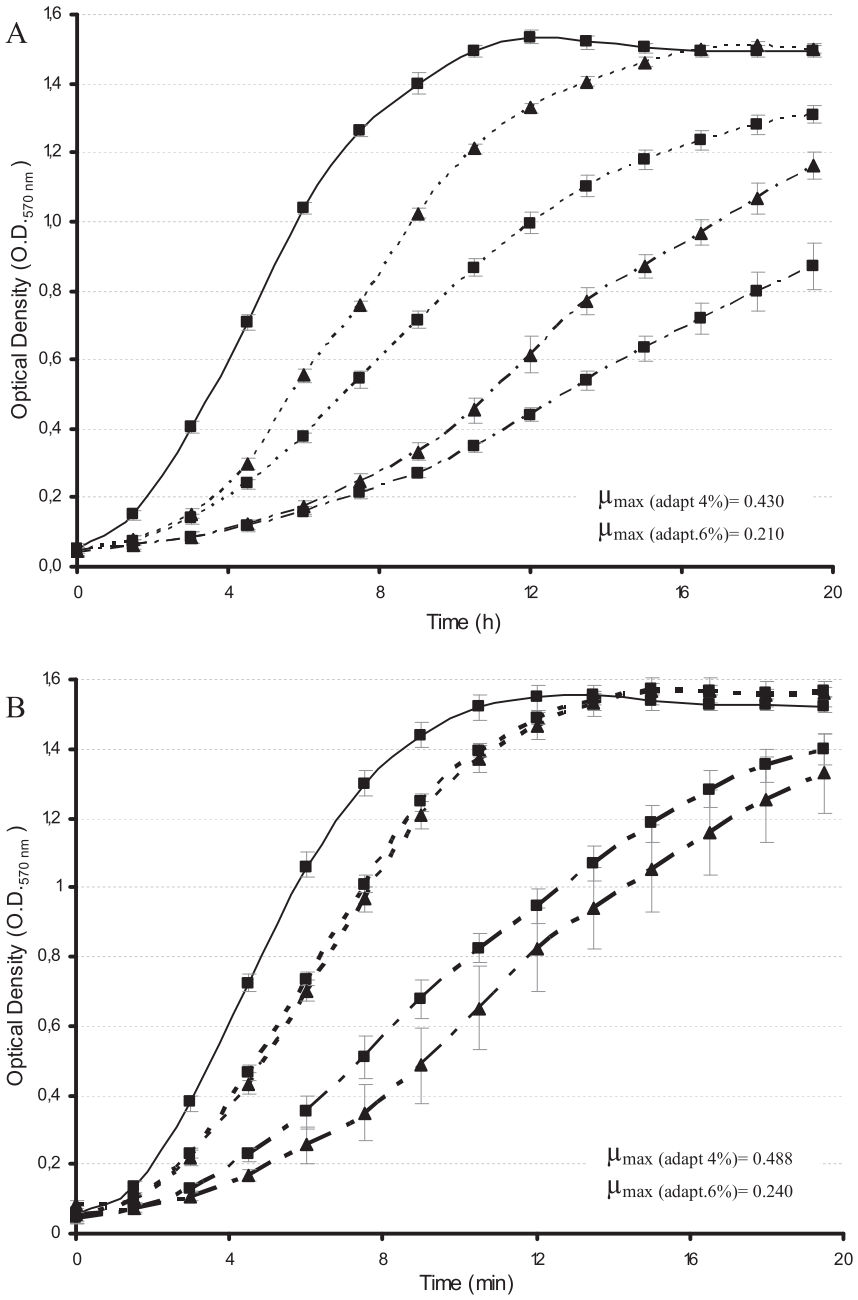

Fig. 7. Growth kinetics at 30 °C (20 h) in MRS broth without (full line, control) and with NaCl 4% (dash line) and 6% (dash-drop line) of Lp 813 (A) and Lp 998 (B), without (■) and with (▲) adaptation (45 °C–30 min). The μ_{max} ($\ln OD_f - \ln OD_0/\theta_f - \theta_0$, OD_f = final optical density; OD_0 = initial optical density; θ_f = final time; θ_0 = initial time) values for the adapted strains are shown in curves.

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