Food Microbiology 48 (2015) 63–71

Contents lists available at ScienceDirect

Food Microbiology

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

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

Verónica Ferrando, Andrea Quiberoni, Jorge Reinhemer, Viviana Suárez*

Instituto de Lactología Industrial (Universidad Nacional del Litoral – CONICET), 1 de Mayo 3250, S3000AOM Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form
12 November 2014
Accepted 16 December 2014
Available online 25 December 2014

Keywords:
Lactobacillus plantarum
Technological stress
Lactic acid bacteria
Cell adaptation

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

* Corresponding author. Tel.: +54 342 4530302; fax: +54 342 4571162.
E-mail address: vivisuar@fiq.unl.edu.ar (V. Suárez).

http://dx.doi.org/10.1016/j.fm.2014.12.005
0740-0020/

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.