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GROWTH/SURVIVAL OF SOME FUNCTIONAL LACTIC ACID BACTERIA UNDER DIFFERENT STRESS CONDITIONS

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Abstract

Lactic acid bacteria (LAB) used as starter cultures in food industry must survive under several stress conditions, including low pH, low water activity, high and low temperatures, oxidative stress, starvation and competition with other species. Also, a high resistance to low pH values and in the presence of bile salts is necessary for probiotic strains, in order to survive in the stomach and intestine, or even to compete with other bacterial groups in this environment and to colonize the gastrointestinal tract (GIT) of the host. The purpose of the present work was to evaluate the growth/survival of 19 LAB strains with biotechnological applications (production of bacteriocins, exopolysaccharides, S-layer proteins) under different stress conditions. The strains were investigated for tolerance to acidity (pH 2.0, 3.5, 4.5 and 5.5), bile salts (0.05%, 0.1%, 0.2%, 0.3% and 0.5%), different temperatures (15°C, 20°C, 28°C, 37°C, 42°C, 47°C, and 50°C), and different concentrations of NaCl (1%, 3%, 5%, and 7%). All 19 tested strains grew well at 28°C and 37°C, with a viability between 10^{10}-10^{14} CFU/ml. Three strains grew well at 50°C reaching a viability of 10^{8}-10^{9} CFU/ml. Four strains were resistant to pH 3.5 and 4.5, after 24 h of incubation reaching a viability of 10^{10} CFU/ml and 10^{14} CFU/ml, respectively. After 3 h of exposure to pH 2.0, two strains, namely Lb. brevis FV403 and Lb. brevis FV530, maintained a viability of 10^{2}-10^{3} CFU/ml. Concerning the bile salt resistance, all tested strains showed a high resistance to a concentration of 0.3% (w/v), reaching a cell viability of 10^{7}-10^{9} CFU/ml after 24 h of incubation. After 3 h of exposure to 0.5% (w/v) of bile salts, the viability of Lb. brevis FV530 was about 10^{10} CFU/ml. Six of the selected strains had a high resistance to NaCl (up to 7%) reaching a viability of 10^{9} CFU/ml after 48 h of incubation.

Key words*: bacteriocins, exopolysaccharides, lactic acid bacteria, S-layer, stress resistance.*

INTRODUCTION

Lactic acid bacteria (LAB) constitute a heterogeneous group of bacteria which are found in diverse environments, such as fermented foods, soil, plants, human and animal body. These bacteria have been used since ancient time to produce various fermented foods from products derived from animals (milk, meat, fish, etc.) or plants (vegetables, wine, olives, sourdough etc.) (Doyle and Beuchat, 2007; Wood, 1997; Stitles, 1996). LAB are also well known for their health-related implications and therefore, they have attracted much attention from scientists. The genetics, physiology and metabolism of LAB have been under rigorous investigation over the past decades (Ganzle, 2015; Pritchart and Coolbear, 1993; Wood and Warmer, 2003).

LAB strains used as starter cultures in food industry are exposed to a variety of stresses, including low pH, low water activity, high and low temperatures, oxidative stress, starvation and competition with other species. In addition, these strains must also resist the adverse conditions encountered in industrial processes, for example during starter handling and storage (freeze-drying, freezing or spray-drying) (Zotta et al., 2008). On the other hand, LAB used as probiotics must endure a number of stresses to ensure they reach the target site in an adequate number to elicit an effect (Mills et al., 2011). Probiotic bacteria must be firstly processed in a suitable form to enable oral consumption. Large-scale manufacture of probiotic bacteria involves growth in large-scale fermentors followed by cooling, harvesting, freezing, and/or drying.

Many recent studies focused on the identification of the stress-sensing systems and defenses against stress in LAB and the

mechanisms of adaptation of these bacteria to harsh environmental conditions (Van de Guchte et al., 2002; Serrazanetti et al., 2009; Mills et al., 2011). Several compounds produced or overproduced by LAB under stress conditions, most probably involved in the protection of producing cells, have been described and characterized (Van de Guchte et al., 2002; Champomier-Verges et al., 2010).

Stress not only induces changes for a better survival, but also different performances in a system, which can be exploited in fermented foods, to get high quality products, with improved sensory and even structural properties (Serrazanetti et al., 2009). Several studies have been focused on the impact of the stress factors on bacteriocin production by LAB (Leroy and De Vuyst, 1999; Neysens et al., 2003; Zamfir and Grosu-Tudor, 2009). Recently, a positive correlation was observed between EPS production and resistance to bile salt and low pH in *Bifidobacterium* species isolated from breast milk and infant feces (Alp and Aslim, 2010). A protective effect of EPS from harsh environmental conditions was proposed by Mills et al. (2011). The same protective effect against hostile factors and the importance for the maintenance of cellular functions has been proposed for S-layers (Mobili et al., 2010). Moreover, S-layer has been proven to have a significant role in the aggregation and adhesion to epithelial cells or to mucus and extracellular matrix proteins, contributing thus to the probiotic activity of the producing cells (Mobili et al., 2010; Golowczyc et al., 2007; Frece et al., 2005). The fermentation medium composition and environmental stresses have been shown to influence the physicochemical surface properties and the S-layer biosynthesis (Schaer-Zammaretti and Ubbink, 2003; Khalegi et al., 2010).

In the context presented above, any new data about adaptation to stress in the case of LAB, especially in the case of strains with functional properties, is very important from a scientific and technological point of view.

The aim of the present study was to investigate the effect of incubation temperature, acidity, the presence of NaCl and bile salts on the growth/survival of some selected LAB strains with functional properties.

MATERIALS AND METHODS

Bacterial strains, media and culture conditions

Nineteen LAB strains from our laboratory collection, isolated from Romanian artisan dairy products, fermented vegetables and other products of plant origin, such as borș or fresh vegetables, were selected for this study. These strains have been previously shown to have functional properties, such as production of bacteriocins (five strains, Grosu-Tudor et al., 2014), exopolysaccharides (nine strains, Grosu-Tudor et al., under evaluation), and S-layer proteins (five strains, unpublished results) (Table 1). The cultures were preserved in MRS broth (de Man et al., 1960) and stored at -80°C in the presence of 25% (v/v) of glycerol as cryo-protectant. Prior to use, the strains were subcultured twice in MRS broth and incubated overnight at 37°C.

Stress treatments

LAB strains were subjected to different stress conditions: incubation temperatures ranging from 15 to 50°C, pH values of the growth medium from 3.5 to 5.5 (pH adjusted with 1N HCl), addition to the growth medium of NaCl up to 7% (w/v), and bile salts (Sigma-Aldrich Chemie GmbH, Germany) up to 0.2% (w/v), respectively. Growth/survival was followed by measuring the pH and the optical density of the culture at 600 nm and by counting the colony forming units (CFU/ml) on a solid MRS medium (1.5% w/v of agar).

Acid tolerance

For the acid tolerance evaluation, three strains resistant to low initial pH values of the growth medium were selected: *Lb. amylolyticus* P40, *Lb. brevis* FV403, and *Lb. brevis* FV530. Two milliliters of overnight cultures of the tested strains were centrifuged (10 minutes at 10000 rpm) and the cellular sediments were resuspended in two ml of MRS broth previously adjusted with 1N HCl to pH 2.0. The cells suspensions were incubated at their optimal temperature for 24 h and samples were taken at different time intervals to determine the viable cell counts (CFU/ml).

Table 1. LAB strains used throughout the study, source of isolation and their functional properties

Strain	Source of isolation	Functional property
Lact. lactis 19.3	cows milk	bacteriocins
Ent. durans 41.2	sour cream	bacteriocins
Lb. amylolyticus P40	borș	bacteriocins
Lb. oris P49	borș	bacteriocins
Lb. amylolyticus P50	borș	bacteriocins
Leuc. mesenteroides P93	cucumber	exopolysaccharides
Leuc. mesenteroides P109	bell peper	exopolysaccharides
Leuc. mesenteroides P112	carrot 1	exopolysaccharides
Leuc. mesenteroides P113	carrot 2	exopolysaccharides
Leuc. mesenteroides P116	green beens	exopolysaccharides
Leuc. mesenteroides P124	yellow beens	exopolysaccharides
Leuc. mesenteroides P127	white cabbage	exopolysaccharides
Leuc. mesenteroides P133	orach	exopolysaccharides
Leuc. mesenteroides P138	lovage	exopolysaccharides
Lb. parabrevis FV196	brine (cauliflower)	S-layer
Lb. brevis FV403	brine (mixed vegetables)	S-layer
Lb. brevis FV530	brine (mixed vegetables)	S-layer
Lb. helveticus RFF 34.9	fermented milk	S-layer
Lb. brevis RFF 46.5	sour cream	S-layer

Bile salts tolerance

In order to determine the bile salts tolerance, three strains resistant to high concentrations (0.2%) of bile salts in the growth medium were selected: *Leuc. mesenteroides* P124, *Lb. brevis* FV403, and *Lb. brevis* FV530, respectively. Two ml of the overnight cultures were centrifuged (10 min at 10000 rpm) and the sediment was resuspended in two ml of MRS broth containing 0.3% and 0.5% bile salts (Sigma-Aldrich Chemie GmbH, Germany), respectively and incubated at optimal temperature for 24 h. Samples were taken at 0, 15, 30, 60, 120, 180 min, and at 24 h of incubation to determine the viable cell counts.

Viable cell enumeration

Viable cells were enumerated by plating 10-fold serial dilutions on MRS agar medium. Plates were incubated at 37°C for 48 h and the LAB counts were expressed in colony forming units per milliliter (CFU/ml).

RESULTS AND DISCUSSIONS

Nineteen LAB strains previously isolated from Romanian fresh/fermented vegetables, dairy products and borș were selected to evaluate the growth/survival under different stress conditions. All these strains might find application in food biotechnology, for the production of functional foods and therefore, it is important to know their capacity to survive under the harsh conditions used in the technological processes. In this context, our study shows the response of some LAB strains with functional properties (EPS biosynthesis, potential probiotic effect and S-layer production) to the stresses induced by low and high incubation temperatures, acidity, NaCl, and bile salts. Cold/heat, salt, and low pH-induced stresses are often encountered during the technological processes in food, while environments with low pH and bile salts are usually encountered during the passage through the human gastro-intestinal tract.

Growth/survival under stress conditions

All LAB strains used throughout this study grew well at 28°C and 37°C, reaching viable cell counts between 10^{10}-10^{14} CFU/ml (Table 2). This was expected, since half of the tested strains belong to *Leuc.* sp. and are mesophilic, with optimal growth temperatures between 20°C and 30°C (Wood and Holzapfel, 1995). Two strains, *Lb. oris* P49 and *Lb. amylolyticus* P50, showed a better growth at 42°C compared with the other tested temperatures, reaching viable cell counts of about 10^{11} CFU/ml. Furthermore, these strains showed a good growth even at a higher temperature, of 50°C, reaching a cell viability between 10^{8}-10^{9} CFU/ml (results not shown). We have

previously shown that *Lb. acidophilus* IBB801, which is a thermophilic strain, still grows at 47°C, but does not grow at temperatures higher than 50 °C (Zamfir and Grosu-Tudor, 2009). Ten out of the 19 tested strains, mostly *Leuc.* species, showed a good growth when they were incubated at 15°C, reaching a cell viability between 10^{10}-10^{12} CFU/ml (Table 2).

Leuconostoc species may naturally adapt to decreases in temperature and they continue to grow after a temperature decrease of about 20°C below the optimum growth temperature (de Angelis and Gobbetti, 2004). Also, *Ent. durans* 41.2 grew well at 15°C reaching a viability of about 10^{10} CFU/ml. Adaptation to low temperature is of vital importance since many fermentations are started by adding frozen or lyophilized starters that would benefit from a high freeze survival capacity. The high viability of this strain during low temperature storage of fermented products prior to consumption is a determinant for probiotic effects.

Table 2. Cell viability (CFU/ml) of the tested LAB strains under different temperatures

Strain	Temperature					
	15°C	**20°C**	**28°C**	**37°C**	**42°C**	**47°C**
Lb. amylolyticus P40	0	0	8×10^9	5×10^9	6×10^9	1×10^8
Lb. oris P49	0	0	5×10^9	7×10^9	1.6×10^{11}	1.4×10^8
Lb. amylolyticus P50	0	0	6.5×10^9	4×10^{11}	3.5×10^{10}	1.8×10^8
Lact. lactis 19.3	0	2.6×10^{12}	1.9×10^{13}	1.4×10^{10}	2.5×10^{7}*	0
Ent. durans 41.2	5.5×10^{10}	2.2×10^{12}	1.2×10^{14}	2×10^{13}	1.1×10^{12}	0
Leuc. mesenteroides P93	5.2×10^{11}	1.03×10^{12}	6.7×10^{12}	2.3×10^{11}	8.2×10^{7}*	0
Leuc. mesenteroides P109	2.5×10^{11}	7.3×10^{11}	1.5×10^{12}	6.8×10^{10}	0	0
Leuc. mesenteroides P112	1.5×10^{12}	1.8×10^{15}	1.05×10^{12}	1.8×10^{12}	1.5×10^{7}*	0
Leuc. mesenteroides P113	9.7×10^{11}	2.9×10^{13}	1.04×10^{13}	9×10^{11}	4.8×10^{6}*	0
Leuc. mesenteroides P116	6.8×10^{11}	1.1×10^{13}	2.3×10^{11}	3.5×10^{11}	1.7×10^{7}*	0
Leuc. mesenteroides P124	1.1×10^{12}	2.2×10^{13}	5.06×10^{12}	5.8×10^{11}	1.3×10^{7}*	0
Leuc. mesenteroides P127	4×10^{12}	9.7×10^{12}*	3.4×10^{12}	4.8×10^{12}	1.5×10^{6}*	0
Leuc. mesenteroides P133	1.4×10^{12}	1.6×10^{14}	2.2×10^{13}	2.2×10^{12}	2.6×10^{6}*	0
Leuc. mesenteroides P138	0	1.5×10^{14}	1.4×10^{14}	4.1×10^{12}	1.1×10^{7}*	0
Lb. parabrevis FV196	0	1.7×10^{13}	1.9×10^{14}	5.4×10^{12}	6.3×10^{11}	0
Lb. brevis FV403	5.3×10^{12}	3.9×10^{12}	6×10^{13}	1.5×10^{12}	1.2×10^{12}	0
Lb. brevis FV530	0	1.0×10^{12}	2.4×10^{12}	5.8×10^{12}	1.4×10^{11}	0
Lb. helveticus RFF34.9	0	5.2×10^{10}*	2.1×10^{12}	8×10^{12}	8×10^{12}	3.6×10^{7}
Lb. brevis RFF46.5	0	3.6×10^{13}	5.4×10^{12}	2.2×10^{12}	2.8×10^{4}*	0

*values measured at 48 h

The ability of the LAB strains to tolerate acid is commonly used as one of the preliminary selection criteria for probiotic candidates.

The effect of initial pH of the growth media was tested for ten selected LAB strains. Three pH values of the growth medium were used, ranging from 3.5 to 5.5. The results showed that resistance to low pH is strain dependent. All ten strains tested were resistant to pH 5.5 showing viability rates of about 10^9-10^{13} CFU/ml after 24 h of incubation (Table 3).

Table 3. Growth parameters at different pH values

Strain	pH 3.5		pH 4.5		pH 5.5	
	OD_{600nm}	**CFU/ml**	**OD_{600nm}**	**CFU/ml**	**OD_{600nm}**	**CFU/ml**
Lb. amylolyticus P40	0.31*	2.6×10^8	4.41*	7.1×10^{10}	3.39	2.1×10^{10}
Lact. lactis 19.3	-	0	1.70*	5.6×10^9	1.35*	2.7×10^9
Ent. durans 41.2	1.61*	4.5×10^{10}	5.98	2.6×10^{13}	8.45	2.5×10^{13}
Leuc. mesenteroides P109	-	0	0.82*	4.2×10^9	1.71	7.7×10^{11}
Leuc. mesenteroides P112	-	0	1.19*	1.5×10^{10}	2.63	4.1×10^9
Leuc. mesenteroides P124	-	0	1.22*	9.1×10^{10}	1.92	9.5×10^9
Leuc. mesenteroides P127	-	0	1.26*	9.2×10^9	2.71	6×10^9
Lb. brevis FV403	1.8	4.3×10^{10}	7.16	4.7×10^{12}	8.52	5.6×10^{12}
Lb. brevis FV530	1.13*	4.5×10^8	3.46	5.7×10^{12}	5.27	4.3×10^{12}
Lb. helveticus RFF34.9	-	0	1.50	1.4×10^9	4.55	4.4×10^{11}

*values measured at 48 h

At pH 4.5, four strains, namely *Ent. durans* 41.2, *Lb. brevis* FV403, *Lb. brevis* FV530 and *Lb. helveticus* RFF34.9, showed a good growth after 24 h of incubation, cell viability reaching a maximum of about 10^{13} CFU/ml. When exposed at pH 3.5, one strain, *Lb. brevis* FV403 showed a good growth after 24 h of incubation, reaching a cell viability of about 10^{10} CFU/ml and other three strains, namely *Lb. amylolyticus* P40, *Ent. durans* 41.2, and *Lb. brevis* FV530, showed a good growth after 48 h of incubation reaching a cell viability between 10^{8}-10^{10} CFU/ml (Table 3). Six strains were not able to grow when they were exposed at pH 3.5. Most probably, the acid regulatory mechanisms of this LAB have failed to maintain their intracellular pH and the internal acidification had reduced the activity of enzymes, damaged certain proteins and DNA, which leads to death (Van De Guchte et al., 2000). Similar results were obtained by Chow and Weimer (1999) on *Lb. acidophilus* strains from the American Type Culture Collection (Rockville, MD).

Bile salts belong to the factors that may significantly affect the viability of LAB in the gastrointestinal tract, influencing the health of the host. Tolerance to bile salts is a prerequisite for colonisation and metabolic activity of bacteria in the small intestine of the host (Havenaar et al., 1992).

After 24 h of incubation in MRS medium supplemented with 0.05% (w/v) of bile salts, all tested strains showed viability rates of at least 10^{7} CFU/ml (Table 4).

Table 4. Cell viability (CFU/ml) of the tested strains under different bile salts concentrations, measured at 24 h of incubation

Strain	**Bile salts**		
	0.05%	**0.1%**	**0.2%**
Lb. amylolyticus P40	3×10^{8}	4.3×10^{6}	5×10^{2}
Lact. lactis 19.3	6.2×10^{8}	1.3×10^{7}	0
Ent. durans 41.2	1.4×10^{12}	9.6×10^{9}	4.4×10^{9}
Leuc. mesenteroides P109	1.9×10^{9}	7.2×10^{7}	1.7×10^{5}
Leuc. mesenteroides P112	3.8×10^{9}	4.7×10^{8}	2.7×10^{5}
Leuc. mesenteroides P124	6.8×10^{9}	1.7×10^{8}	4.4×10^{5}
Leuc. mesenteroides P127	2×10^{9}	2×10^{8}	3×10^{5}
Lb. brevis FV403	4.5×10^{11}	6.2×10^{9}	1×10^{9}
Lb. brevis FV 530	5.7×10^{9}	4.7×10^{8}	6.1×10^{5}
Lb. helveticus RFF43.9	1.9×10^{7}	2×10^{2}	0

At higher concentration of bile salts (0.1%, w/v), nine out of ten strains showed a good growth, reaching a viability between 10^{6}-10^{9} CFU/ml. A variable resistance to higher concentration of bile salts (0.2%, w/v) was observed for the tested strains. This concentration of bile salts did not allow the growth of two strains, *Lact. lactis* 19.3 and *Lb. helveticus* RFF 34.9, respectively (Table 4). Seven strains proved a long-term resistance at this bile salts concentration, reaching viability rates between 10^{5}-10^{9} CFU/ml. This high resistance to bile salts represents an advantage for the survival of these bacteria in the gastrointestinal tract. Also Zamfir and Grosu-Tudor (2013) found high tolerance to 0.2% of bile salts for a strain isolated from dairy products, namely *Lb. acidophilus* IBB801.

LAB are often exposed to changes in the solute concentrations of their natural habits (De Angelis and Gobbetti, 2004). Nevertheless, their cytoplasmic solute concentration needs to be relatively constant and therefore, LAB needs to develop some defense mechanisms. A sudden increase in the osmolarity of the environment (hyperosmotic stress) results in the movement of water from the cell to the outside, which causes a detrimental loss of cell turgor pressure, changes in intracellular solute concentration and changes of the cell volume (Poolman and Glaasker, 1998).

All tested strains showed a very good tolerance to 1% NaCl, reaching viability rates between 10^{9}-10^{13} CFU/ml (Table 5). After 24 h of incubation, in the presence of 3% NaCl, eight out of nine tested strains reached a viability of maximum 10^{12} CFU/ml. In the presence of the

highest concentration of NaCl (7%, w/v), two strains, *Lb. brevis* FV403 and *Lb. brevis* FV530, reached a viability of about 10^8 CFU/ml after 24 h of incubation. The high resistance of these two strains was expected since they were isolated from samples collected from fermented vegetables which contain a high concentration of salt (Wouters et al., 2013). The observed differences in osmotic rezistance of different bacterial strains may be due to the distinct composition of their membrane phospholipids. Several works have shown a relationship between membrane stability and the ability of cells to resist osmotic stress (Linders et al., 1997; Laroche and Gervails, 2003).

Table 5. Cell viability (CFU/ml) of the tested strains under different NaCl concentrations

Strain	0% NaCl	1% NaCl	3% NaCl	5% NaCl	7% NaCl
Lb. amylolyticus P40	3.6×10^9	1.4×10^9	0	0	0
Lact. lactis 19.3	5.9×10^{11}	9.9×10^9	9.1×10^9	2.7×10^5*	0
Leuc. mesenteroides P109	1.1×10^{12}	1.8×10^{11}	1.6×10^{10}	5.2×10^9	1.2×10^9*
Leuc. mesenteroides P112	3.3×10^{12}	5.3×10^{11}	1.8×10^{11}	1.2×10^{10}	7.5×10^7*
Leuc. mesenteroides P124	2.4×10^{11}	2.4×10^{12}	6.4×10^{12}	3×10^9	8.5×10^7*
Leuc. mesenteroides P127	1.1×10^{12}	1.5×10^{13}	4.6×10^{12}	1.6×10^{10}	7.5×10^7*
Lb. brevis FV403	1.5×10^{11}	1.9×10^{12}	4.9×10^{10}	9.9×10^{11}	8.7×10^8
Lb. brevis FV530	5×10^9	2.2×10^{10}	1×10^9	5.5×10^9	2.4×10^8
Lb. helveticus RFF34.9	7.5×10^9	8.3×10^9	6.2×10^6	0	0

*values measured at 48 h

Acid tolerance

The best acid tolerance was shown by two strains, namely *Lb. brevis* FV403 and *Lb. brevis* FV530. After 2 h of incubation at pH 2.0, the viable cell counts of these two strains decreased with 9 and 6 logs, respectively, as compared with the initial values (Figure 1). This was probably to better pH regulation ability as compared with the other strains. Our results are in accordance with Young et al. (2005) who showed a higher survival of *Lb. brevis* strains under low pH conditions as compared to other *Lactobacillus* species. Similar results were obtained by Pato (2003) for some LAB strains isolated from "dadih", an Indonesian fermented food.

After 3 h of incubation at pH 2.0, the viability of the cells was completely lost. Also, Young et al. (2015) described a lethal effect of pH 2.0 environment on some selected *Lactobacillus* strains after 4 h of incubation at 37°C. The reduction of survivability of LAB strains at low pH was also reported by Raghavendra et al. (2010), for strain *Pediococcus pentosaceus* CFR R123 (53%) and *L. rhamnosus* GG (55%) after incubation at pH 2.0 for 3 hours.

For *Lb. amylolyticus* P40, the viable cells concentration decreased very fast, after 1h of incubation being with 5 logs than the control, while after 2 h the viability was completely lost (Figure 1).

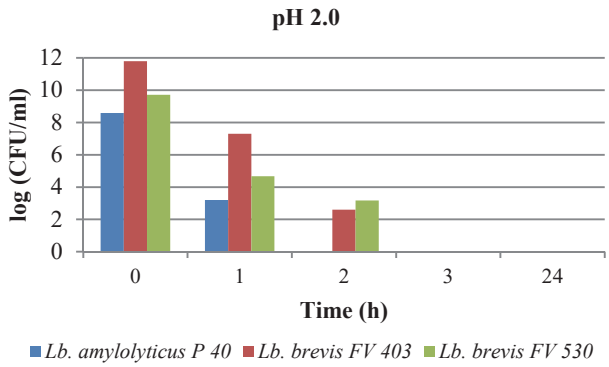

Figure 1. Acid tolerance of the selected LAB strains at pH 2.0

Bile salts tolerance

Bile salts resistance is important since it helps LAB to reach the small intestine and colon and contribute in balancing the intestinal microbiota.

The survival rates of all tested LAB strains decreased with the increase of bile salts concentration. However, the *Lactobacillus* strains were generally more tolerable to low concentration of bile salt (0.3%, w/v), reaching viability rates of about 10^7 CFU/ml after 24 h of incubation (Figure 2).

A similar decrease in viable counts was also detected by Young et al. (2015) for some LAB strains isolated from Malaysian fermented

Bambangan (*Mangifera paiang*) and by Wang et al. (2010) for three *Lactobacillus* strains obtained from infant faeces and pickled vegetables.

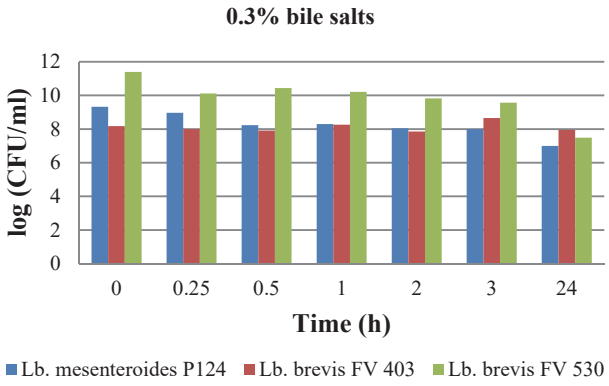

Figure 2. Resistance of the selected LAB strains in the presence of 0.3% bile salts

In the presence of higher concentration of bile salts (0.5%, w/v), one strain, *Lb. brevis* FV530, proved a good long-term tolerance, the viable cell counts decreasing with only 2 logs as compared with the initial value (Figure 3). This strain was found to produce S-layer proteins (Grosu-Tudor et al., 2016).

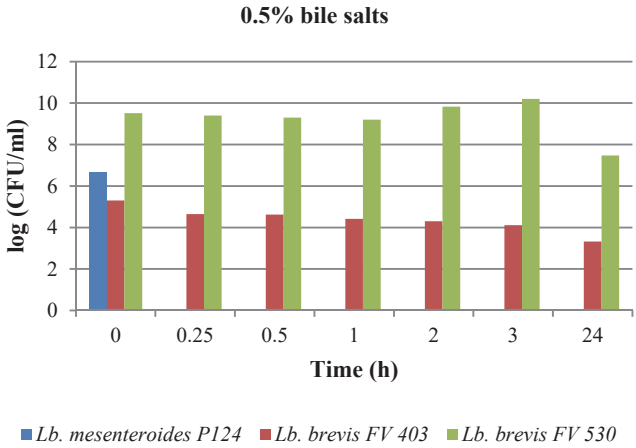

Figure 3. Resistance of the selected LAB strains in the presence of 0.5% bile salts

Among others, these proteins have important cell functionality, such as acting as protective barrier against environmental hazards, controlling the transfer of nutrients and metabolites etc. (Buck et al., 2005).

Bile salt tolerance is considered one of the essential properties required for LAB to survive in the small intestine (Sarela et al., 2000). Succi et al. (2005) found similar results on *Lb. rhamnosus* strains isolated from Parmigiano Reggiano cheese, which showed a good survival in the presence of 1%, 1.5% and 2% bile salts.

The survival ability of these LAB strains in the stress conditions mentioned above might result in a temporary colonization of the human gastro-intestinal tract (Alander et al., 1997; Johansson et al., 1998).

CONCLUSIONS

In conclusion, our study highlights the diversity of stress response in some LAB with functional properties. *Lb. brevis* FV530, isolated from Romanian fermented vegetables was the most tolerant strain to low pH and high concentration of bile salts, which makes it suitable for further application as a probiotic strain.

Also, this strain may be used as adjunct culture or incorporated into an industrial process due to its tolerance to the environmental stress such as elevated temperatures and high salt concentrations.

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