Stress response of some lactic acid bacteria with bionanotehnological applications

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Lactic acid bacteria (LAB) constitute a heterogeneous group of industrially important bacteria that are used to produce fermented foods and beverages. LAB are also well known for their health-related implications and therefore, they have attracted much attention from scientists. LAB strains used as starter cultures are exposed to a variety of stresses, including low pH, high/ low temperatures, oxidative or osmotic stress, starvation etc., while LAB used as probiotics need to survive in the digestive tract of the host (low pH, pancreatic enzymes, bile salts, low amounts of nutrients), and express their specific functions in these unfavorable conditions.

The objective of the proposed project is to study the influence of various stress conditions on the growth/survival, metabolism and structure of some LAB strains with bionanothechnological impact (strains producing bacteriocins or exopolysaccharides, strains with probiotic potential, and strains producing S-layer proteins). Moreover, their ability to produce active compounds, with unaltered properties, in these stress conditions, will be investigated. The changes induced by stress will be evaluated using different approaches, including: microscopical observations, biochemical determinations, and molecular studies. Finally, some strategies for inducing stress tolerance will be looked for (such as cross protection, addition of some compounds able to protect the culture against the stress conditions etc.).

The first task of the project is: **„Selection of lactic acid bacteria with bionanotehnological application to be used in the project"**

In this first task, the LAB strains to be used during the project were selected based on their ability to produce compounds with bionanotechnological impact (bacteriocins, exopolysaccharides and S-layer proteins). The culture collection of our department harbours an impressive number of LAB strains isolated mostly from traditionally fermented foods (dairy products, fermented vegetables and cereals-"bors") but also from fresh fruits and vegetables collected from different regions of Romania. During our previous studies, some of the newly isolated strains proved to be of great potential for food biotechnology (bacteriocin-

or exopolysaccharide- producing strains) or with potential health benefit (pro- or prebiotic effect). Some of these strains were selected for further studies concerning their stress response. Additionally, some strains were chosen based on their ability to produce S-layer proteins, after a screening based on electrophoretic methods.

The selected strains were listed in table 1.

Table 1. Lactic acid bacteria strains selected to be used in this project, their source of isolation and their bionanotehnological compounds

Strain	**Source of isolation**	**Bionanotehnological compounds**
Lactococcus lactis 19.3	Fersh caw milk	Bacteriocins
Enterococcus durans 41.2	Fermented milk (sour cream)	Bacteriocins
Lactobacillus amylolyticus P40	Fermented cereals (borş)	Bacteriocins
Lactobacillus oris P49	Fermented cereals (borş)	Bacteriocins
Lactobacillus amylolyticus P50	Fermented cereals (borş)	Bacteriocins
Lactobacillus mesenteroides P93	Fresh vegetables (cucumber)	Exopolysaccharides
Lactobacillus mesenteroides P109	Fresh vegetables (bell peper)	Exopolysaccharides
Lactobacillus mesenteroides P112	Fresh vegetables (carrot 1)	Exopolysaccharides
Lactobacillus mesenteroides P113	Fresh vehetables (carrot 2)	Exopolysaccharides
Lactobacillus mesenteroides P116	Fresh vegetables (green beens)	Exopolysaccharides
Lactobacillus mesenteroides P124	Fresh vegetables (yellow beens)	Exopolysaccharides
Lactobacillus mesenteroides P127	Fresh vegetables (white cabbage)	Exopolysaccharides
Lactobacillus mesenteroides P133	Fresh vegetables (orach)	Exopolysaccharides
Lactobacillus mesenteroides P138	Fresh vegetales (lovage)	Exopolysaccharides
Lactobacillus parabrevis FV196	Fermented vegetables	S-layer proteins
Lactobacillus brevis FV403	Fermented vegetables	S-layer proteins
Lactobacillus brevis FV530	Fermented vegetables	S-layer proteins
Lactobacillus helveticus RFF34.9	Fermented milk	S-layer proteins
Lactobacillus brevis RFF46.5	Fermented milk	S-layer proteins

The first two **bacteriocins** used in this study are heat stable, low-molecular mass peptides, with a wide inhibitory spectrum (including *Listeria monocytogenes* and *Staphylococcus aureus*), while the other three are heat sensitive, high-molecular mass proteins, with a very narrow inhibitory spectrum. Bacteriocin production can be considered as

an advantage and a functional role for LAB strains to be used in the food industry, to improve food quality and safety (De Vuyst and Leroy 2007; Parada et al. 2007). Moreover, bacteriocin production by probiotic LAB may play an important role during *in vivo* interactions occurring in the human gastrointestinal tract (De Vuyst et al. 2004).

The **exopolysaccharides (EPS)** used in this study are homopolysaccharides, composed of glucose, and they have a high molecular mass, over 1.4 MDa. Some of the selected strains produces considerable amounts of EPS (over 25 g/l). EPS produced by lactic acid bacteria can be used in the fermented dairy industry because of their potential applications as viscosifiers, texturizers, and emulsifying agents (Grobben et al., 1996). They also possess antitumoral (Ebina et al., 1995; Oda et al., 1983), immunostimulatory (Hosono et al., 1997), macrophage (Nishimura-Uemura et al., 2003), and lymphocyte (Kitazawa et al., 1998) activating activities, they enhance the colonization of the gastrointestinal tract by probiotic bacteria and act as antioxidants (Badel et al., 2011; Hugenholte & Smid, 2002; Polak-Berecka et al., 2013). Regarding their physiological role, EPS from LAB have been claimed to protect cells from detrimental environmental conditions, such as dehydration, macrophages, antibiotics, and bacteriophages, to sequester essential cations, and to be involved in adhesion and biofilm formation (Looijsteijn et al., 2001).

The **S-layer proteins** gave a dominant band in SDS-PAGE and the extraction procedure was monitored by using this method. Also the presence of S-layer proteins covering the cells was detected as a light layer surrounding the cells through transmission electron microscopy (TEM). These proteins are involved in important cell functionalities such as acting as a protective barrier against environmental hazards, controlling the transfer of nutrients and metabolites, maintaining the cell shape and envelope rigidity, promoting cell adhesion and surface recognition, among others (Vidgrén et al. 1992; Buck et al. 2005).

The **main objective** of this task was to evaluate the ability of the selected strains to grow/survive under various conditions (different temperatures, pH values of the growth media, the addition of bile salts or NaCl to the growth media), in order to establish the optimal and the stress conditions to be further used. The growth was followed in time by spectrophotometrical and pH determinations, and by counting the viable cells (CFU/ml).

The experimental results obtained in the final stage of this project in 2015 led us to the following conclusions:

- Lactic acid bacteria strains showed a high resistance to low pH, they can survive to acidic conditions in the stomach for at least two hours.

- Also, two strains of lactic acid bacteria tested, *Lactobacillus brevis* FV 403 and *Lactobacillus brevis* 530 are rezistant to bile salt in the presence of at least 0.5%
- Siw of the selected strains had a high resistance to up to 7% NaCl. Adaptation of the tested strains to high concentration of NaCl may be an advantage in their used in the food industry in order to obtain food with improved properties because the technological process involves most of the time addition of NaCl.

The objectives proposed for this stage were fullfiled.

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