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ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2021; 26(2): 2548-2559
doi: 10.25083/rbl/26.2/2548.2559

Received for publication, February, 4, 2020
Accepted, November, 30, 2020

Original paper

Environmental stress responses in yeasts and lactic acid bacteria strains isolated from dairy traditional Romanian fermented products

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Abstract

During last decades, there is a growing interest for characterizing new microbial strains isolated from various sources (plants, soil and natural fermentative processes), in order to enhance industrial productivity. The aim of the present study was to assess the profile of cell growth parameters and biomass accumulation of 15 newly isolated yeast and lactic acid bacteria (LAB) strains from Romanian spontaneous fermented dairy products under different environmental stress conditions (chemical and physical). On this purpose, the yeast and LAB strains were characterized and identified using MALDI-TOF MS and selected for their biotechnological potential. Cell growth was evaluated in presence of extreme pH values, temperatures and different NaCl concentrations. All strains included in this study grew well under their optimal conditions; some of them preferred extreme parameters: acid / very alkaline pH, high temperatures or NaCl concentration The characterization of microbiota from Romanian spontaneous fermented dairy products might represent a great opportunity for the development of dairy industry using native microorganisms, preserving thus the Romanian biodiversity and cultural heritage.

Keywords Romanian fermented dairy products, osmotolerant microorganisms, extreme pH, heat shock resistant microorganisms.

To cite this article: CORBU V, PETRUT S, VASSU T, PELINESCU D, SARBU I, RUSU E, CSUTAK O. Environmental stress responses in yeasts and lactic acid bacteria strains isolated from dairy traditional Romanian fermented products. *Rom Biotechnol Lett*. 2021; 26(2): 2548-2559. DOI: 10.25083/rbl/26.2/2548.2559

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Introduction

The production of fermented dairy products through spontaneous fermentation has been used for thousands of years and remains until now one of the main methods used for milk processing and preservation. Spontaneous fermentation allows microbial natural selection by generating a stressful environment in which only those microorganisms that have undergone the necessary metabolic adaptations survives. This process is mainly governed by yeasts belonging to various genera and species, such as: *Kluyveromyces lactis, Kluyveromyces marxianus* (YU, 2011), *Saccharomyces cerevisiae, Saccharomyces boulardii, Pichia kudriatzevii, Yarrowia lipolytica, Debaryomyces hansenii, Candida parapsilosis, Candida tropicalis, Kazachstania unispora* (QUIGLEY, 2011; AKABANDA, 2013) and/or lactic acid bacteria (LAB) of which the most representative strains belong to *Lactococcus (Lc.) lactis, Lactobacillus (Lb.) casei, Lb. delbruecki, Lb. acidophilus, Lb. rhamnosus, Lb. helveticus, Lb. plantarum, Streptococcus (St.) thermophilus* (AKABANDA, 2013). The dairy products are classified in two categories, according to the major group of fermentative microorganisms isolated: (i) milk products obtained through lactic fermentation in which LAB strains occupy the central position and (ii) fungal-lactic fermented products where LAB and yeasts act simultaneously to generate the final product (TAMANG, 2016).

Studies on the dynamics of the microbial population found in spontaneously fermented dairy products have revealed that starter LAB are mainly involved in the acidification while yeasts, molds and other non-starter LAB contributing to the organoleptic properties of the products conferring different flavors or textures. Also, some of the microorganisms involved can release beneficial compounds for human health such as: conjugated linoleic acid (CLA) with antioxidative and anti-inflammatory activity and useful for atherogenesis prevention (CHINNADURAI, 2013; PENEDO, 2013); exopolysaccharides (EPS), carbohydrate polymers with positive impact on the human gut microbiota and the immune system (CAGGIANIELLO, 2016); bioactive peptides (peptides mainly derived from casein) that might have antimicrobial, antioxidative, antihypertensive and immunoregulatory activity (FERNÁNDEZ, 2015; HERNÁNDEZ-LEDESMA, 2011); vitamins (folic acid, biotin, cobalamina.s.o) (PATRING, 2006; SAUBADE, 2017); γ-aminobutyric acid (GABA- non-protein amino acids) considered to be highly valuable for pharmaceuticals having hypotensive, diuretic and antidiabetic properties (DHAKAL, 2012; HUDEC, 2015; LIM, 2017) and oligosaccharides (prebiotics) that positively influence growth of bifidobacteria (CHEN, 2017; PADILLA, 2015).

The study of the microbiota involved in spontaneous fermentation is of particular importance from several points of view. First of all, it allows isolation of microorganisms that possess specific metabolic adaptations due to the environment influence and, subsequently, can be used to improve different industrial processes without resorting to genetic engineering techniques. Also, traditional/spontaneous fermentation occurs without sterilization that involves a continuous transfer of microorganisms between the processing environment and the fermented product. This represents an interesting point of view since analyzing the microbiota of the fermented products can reflect the microbiota of the environment from specific geographical areas (QVIRIST, 2016).

In Romania, traditionally fermented dairy products represent an important part of the daily diet and can be developed into an international business card reflecting the local specificity and protecting biodiversity (FEUTRY, 2012; ZAMFIR, 2006). The Romanian dairy market, particularly the organic dairy market, is constantly growing, currently reaching 1.3 million euros. Although some of the market leaders have Romanian origins and the production factories are located on the territory of Romania, the microbial strains used in food fermentations are usually from international collections consisting of microbial strains previously isolated from various geographical regions from aboard (JUNGERSEN, 2014). This is mainly due to the limited interest for the characterization of the native microbial biodiversity specific for spontaneous fermented dairy products that can provide unlimited resources and are more suitable for the needs of the inhabitants of this area. Of course, in this context, the interest of the Romanian entrepreneurs for developing the research segment of their businesses represents an important issue.

During food industrial and biotechnological processes, the microorganisms are exposed to different physical and chemical stress conditions involving temperature shocks, extreme pH and high osmotic pressure induced by high carbohydrates or NaCl concentrations (LÓPEZ-GONZÁLEZ, 2018). Thus, the main objective of this study represents the characterization of biomass accumulation for 15 newly isolated yeast and LAB strains from traditional Romanian fermented dairy products grown under various stress conditions.

Materials and Methods

Microbial strains

A total number of 15 microbial strains, seven yeast strains and eight LAB strains, were isolated from spontaneous fermented milk products from different counties of Romania (Ialomița, Dâmbovița, Bistrița-Năsăud, Ilfov and Mureș) (Table 1).

Table 1. The 15 isolates and isolation source

Strain		Source of isolation	County/Region
YEASTS	Y-L3S	Fermented milk	Ilfov
	Y-DA1	Fermented milk	Ilfov
	Y-SM3	Sour-cream	Bistrita
	Y-CMGB 233	Fermented milk	Ialomița
	Y-CMGB 234	Cheese	Ialomița
	*I.orientalis*CMGB 224*	Yogurt	Ialomița
	*I.orientalis*CMGB 225*	Yogurt	Ialomița
LAB	LAB-Lb20	Fermented milk	Mureș
	LAB-Bz1	Cheese	Dâmbovița
	LAB-U4	Fermented milk	Dâmbovița
	LAB-SM2	Sour-cream	Bistrița
	LAB-P4	Fermented milk	Dâmbovița
	LAB-Bz6	Cheese	Dâmbovița
	LAB-L4	Fermented milk	Dâmbovița
	LAB-S2b	Sour-cream	Dâmbovița

*CMGB – Collection of 81 Microorganisms of the Department of Genetics, Faculty of Biology, University of Bucharest

Purification of the strains was done by streaking on YPGA media (0.5% yeast extract, 1% peptone, 0.2% glucose, 2% agar) for yeasts and MRSA (2% glucose, 1% peptone, 0.5% meat extract, 0.5% yeast extract, 0.2% dipotassium hydrogen phosphate, 0.1% Tween 80, 0.2% diammonium citrate, 0.5% sodium acetate tri-hydrate, 0.01% magnesium sulfate heptahydrate, 0.005% manganese sulfate tetrahydrate, 1.5% agar, pH = 6.5) for LAB strains. After purification, the strains were stored at -70°C in a Revco LegaciTM Refrigeration System (Copeland, U.K) in YPG, respectively MRS broth supplemented with 20% (v/v) glycerol added as cryoprotectant, until further experiments. Prior to the beginning of the experiments, each yeast strain was sub-cultured on YPGA slants for 24 hours at 28°C, while bacterial strains were sub-cultured (1% v/v) in MRS broth for 24 h at 37°C.

Bruker MALDI-TOF identification

For MALDI-TOF MS analysis samples were prepared by resuspending single yeast/bacterial colonies grown on YPGA/MRSA media for 24 h at 28/37°C, in a matrix solution provided by the manufacturer and subsequently applied to a coded plate. MALDI-TOF MS measurements were acquired according to the manufacturer instructions using a Microflex III instrument (Bruker Daltonik, Bremen, Germany) and compared to the reference mass spectra using MALDI BioTyper Software (Bruker Daltonics). The results were expressed as suggested by the manufacturer using scores ranging from 0 to 3. Only scores higher than 2 were taken into account as being reliable for microbial identification (IONESCU, 2015; ÁLVAREZ-BUYLLA, 2012).

Growth at different pH values

Yeast and LAB strains ability to grow under stress conditions induced by different pH values (3-12) was determined using YPG/MRS broth with pH adjusted using a solution of HCl 2N (for pH<6) or NaOH 40% (for pH >6). Overnight yeast and LAB cultures were centrifuged at 7000 rpm for 7 min., washed twice with distilled sterile water and the cell suspensions were adjusted to an OD 600 nm = 1 using a spectrophotometer (VilberLourmat). This suspension was used to inoculate 300 µL of pH adjusted YPG/MRS broth to a final concentration of 1% (v/v). Cell growth was determined by measuring the OD600nm after 24 h of incubation at 28/37°C using a Synergy HTX Multi-Mode Reader (Bio-Tek) (PETRUT, 2016). The positive controls were represented by YPG/MRS broth, that have an initial pH of 6.2 respectively, 6.5.

Growth in osmotic stress conditions

Dairy food processing is most often associated with exposure of microbial cells to osmotic stress. In order to evaluate the ability of our strains to resist to this kind of stress we used YPG/MRS broth with several NaCl concentrations (0.5%; 2%; 5%; 8%; 10%; 12%). After inoculation of the specific culture media as described above, the OD 600 nm was determined after 24 h of incubation at 28/37°C. Positive controls were used to validate the results represented by YPG/MRS broth without NaCl.

Growth at different temperature values

The processes associated with the food industry, usually involve quite large variations in the temperature of the culture media. The ability of yeast and LAB strains to grow at different temperature values (15°C; 22°C; 28°C; 37°C; 42°C; 60°C) was tested by inoculating the yeast/LAB strains in YPG/MRS broth using the same protocol as previously. The microbial growth was monitored by reading the OD 600 nm after 24 h of incubation

(QVIRIST, 2016) and the results were reported to positive controls for which the incubation temperature was 28°C for yeasts, respectively, 37°C for LAB strains.

Statistical analysis

All the experiments were done in triplicates and the results obtained were expressed as mean ± SD (n=3) using Excel tool from Microsoft Office 2016 package.

Results and Discussions

Yeast and LAB strains isolation and identification

Naturally fermented dairy products continue to be a matter of special interest due to the rich microbial biodiversity, which represents a valuable source of microorganisms harboring. A total number of 15 microbial strains isolated from spontaneous fermented dairy products from different regions of Romania were selected for our study. The strains were identified using Bruker MALDI-TOF Identification System, as previously described. The advantage of Bruker MALDI-TOF Identification System is that it can be used to identify a large number of species or species groups, most of them being characteristic for clinical microbiology laboratories. In this study, we were able to identify 13 yeast and LAB strains (Table 2).

Table 2. MALDI-TOF MS identification of the 15 isolates

Strain		MALDI TOF MS identification
YEASTS	Y-L3S	*Candida krusei*
	Y-DA1	*Candida parapsilosis*
	Y-SM3	*Candida krusei*
	Y-CMGB 233	*Hansenula(Ogataea) polymorpha*
	Y-CMGB 234	*Saccharomyces cereviae*
LAB	LAB-Lb20	*Lactobacillus rhamnosus*
	LAB-Bz1	*Lactobacillus plantarum*
	LAB-U4	*Enterococcus faecalis*
	LAB-SM2	*Lactobacillus plantarum*
	LAB-P4	*Lactobacillus paracasei*
	LAB-Bz6	*Lactobacillus plantarum*
	LAB-L4	*Enterococcus faecalis*
	LAB-S2b	*Lactobacillus plantarum*

Regarding the yeast species isolated, three of them were identified as *Candida krusei*. Many studies debate the taxonomic classification of the members of this species. First, it was associated with *P. kudriavzevii* (*I. orientalis*) being considered its anamorphic form. Later, other studies suggested that members of these two species cannot be separated through genome sequencing (DOUGLASS, 2018). However, conventional identification techniques question these assumptions due to a number of differences occurring between the two species, such as the ability to produce ascospores a.s.o (KURTZMAN, 2011). As a consequence, in the present study we will keep the name generated using the MALDI-TOF system, i.e. *Candida krusei.*

The other yeast strains isolated were determined as belonging to *C. parapsilosis, Saccharomyces cerevisiae* and *Hansenula (Ogataea) polymorpha* species. Except *H. (O.) polymorpha*, all these species were previously identified in fermented products and have been used in the dairy industry over time. Therefore, *H. (O.) polymorpha* can be considered an exception because its main isolation sources are soil, orange juice and sometimes clinical samples (MANFRÃO-NETTO, 2019).

The eight newly isolated LAB strains, previously characterized (morpho-physiological and biochemical – data not shown) were taxonomically classified into *Lactobacillus* and *Enterococcus* genera (Table 2). The genus *Lactobacillus* includes the highest number of GRAS (Generally Recognized as Safe) species, many of them having great importance in processes from food industry and human nutrition (SALVETTI, 2012). On the other hand, species belonging to Enterococcus genus are suitable as starter cultures for food biotechnology, with an important role in the development of flavor for different products and other organoleptic characteristics of various fermented foods (PETRUT, 2019).

Assessment of growth parameters profile in different environmental stress conditions

When selecting strains for food fermentation, different aspects, including safety, functional and technological characteristics, have to be taken into consideration (TAMANG, 2016; ZHONG, 2016). Developing new starter cultures is not easy. It is essential to undergo a complex characterization (phenotypic and genotypic) of the strains that are going to be used at industrial scale. Although many microorganisms have great organoleptic

properties, some of them cannot resist to the manufacturing process which implies huge mechanical, thermal, osmotic stress and also pH changes or metabolite induced stress (KANDASAMY, 2018). In order to resist to all these challenges, functional microorganisms have developed different mechanisms of adaptation to this environment.

Growth at different pH values

During the biotechnological processes, such as milk fermentations, yeasts and bacteria used as starter cultures are exposed to a wide range of stress conditions, among which pH fluctuations. The ability to grow under these conditions represents an important technological characteristic of these strains, assuring thus their success in the competition with other groups of microorganisms which co-exist in this environment (LARANJO, 2017; AHMED, 2006). Similarly, yeast and LAB strains used in probiotic products must be able to survive in presence of various pH values characteristics to the gastrointestinal tract (GIT), in order to colonize the GIT of the host and to exert their beneficial properties (PETRUT, 2016; NEMSKA, 2019). Also, resistance to low pH values is extremely important for biofuel production using food industry wastes since many microbial strains can be used for converting starch to glucose without chemical pre-treatment and during this process low pH values are frequently encountered (CHAUDHARY, 2017).

Yeast growth is more active at lower pH values compared to neutral or alkaline pH. According to Figure 1 our yeast strains have an optimal pH growth value in the range 4.0-8.5. Some differences occurred regarding the resistance of *Issatchenkia orientalis* strains to extreme pH conditions. Thus, the strain *I. orientalis* Y-CMGB 225 presented high values of growth at pH 3.0 while the strain *I. orientalis* Y-CMGB 224 presented significant growth at alkaline pH. Also, the strains *Candida krusei* Y-L3S and Y-SM3 grew very well at pH 3.0. Significant growth was also recorded for *S. cerevisiae* CMGB-234 with an optimal pH interval between 4.0 and 8.5. Extracellular pH variation affects yeast cell cycle and viability by influencing pH homeostasis. Optimal internal pH maintenance is mainly mediated using cells buffer systems that allow H+ consumption (BRANDÃO, 2014)/extrusion through plasma membrane H+-ATPase (ARIÑO, 2010). Many studies have focused on the characterization of metabolic pathways involved in pH resistance in yeasts, based mainly on promoting expression of specific genes such as IoGasI (*I. orientalis*) or ScGasI (*S. cerevisiae*). These genes encode glycosylphosphatidylinositol (GPI)-anchored proteins involved in protecting cells wall integrity when exposed to environmental stress (WADA, 2020). *I. orientalis* is considered a multiple stress-tolerant yeast, the members of this species showing great variability of stress condition resistance (MATSUSHIKA, 2016). Due to its metabolic versatility, numerous studies have focused on the utility of *Issatchenkia* species in industrial processes involving high variations of pH values (TOIVARI, 2013).

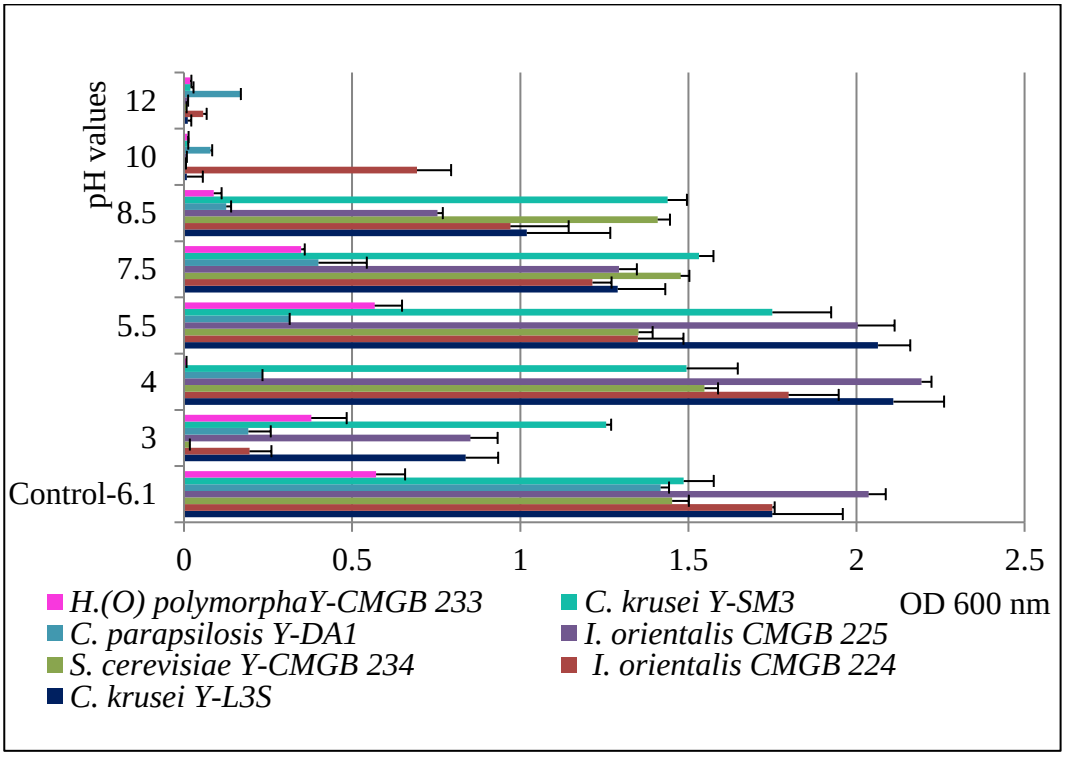

Figure 1. Effect of pH variation on yeast growth.

The results obtained revealed different responses of LAB strains to low/ high initial pH values. All tested strains had an optimal growth at pH range between 5.5 and 7.5, but there were also some strains that grew well at extreme pH values – acid or alkaline (L4 and U4 – pH 10.0 to 12.0; SM2 – pH 4.0). The analysis of the growth curves of tested LAB strains, showed that Enterococcus strains LAB-L4 and LAB-U4 have high resistance to pH 10.0 and pH 12.0, with a maximum OD 600 mn of 1.5, compared to the other strains that registered a significant reduction of the growth rate (Figure 2).

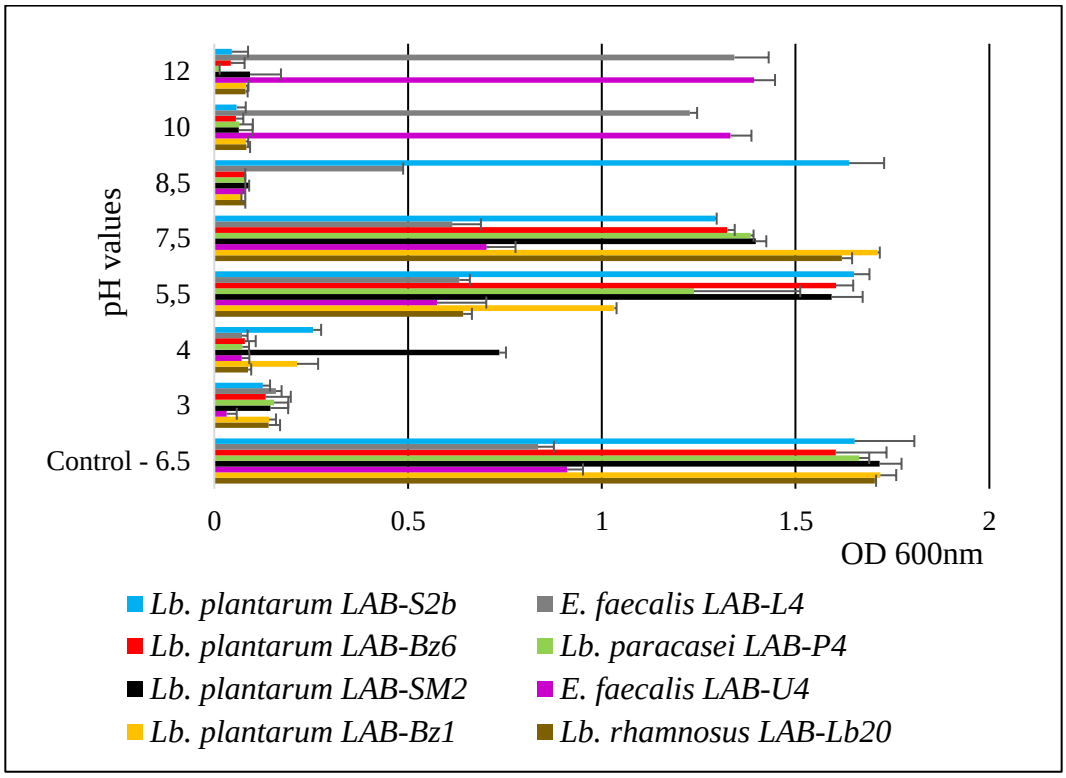

Figure 2. Effect of pH variation on LAB growth.

In contrast, one particular strain, *Lactobacillus plantarum* LAB-SM2, show preferential growth at acid pH 4.0 (OD 600 mn = 0,735) (Figure 2).

The survival of LAB strains in acidic environments has an important impact on health as well as on the economy. Intrinsic resistance to acidic conditions is a relatively rare ability, but there are studies showing species of *Lactobacillus* and *Bifidobacterium* genera are among the most resistant to such conditions (COTTER, 2003; NEMSKA, 2019). The mechanisms used by LAB to survive at low pH stress conditions are very different, three main systems being described as involved in pH homeostasis: H+ - ATPase proton pump, ADI (arginine deaminase) and GAD (glutamate decarboxylase) systems (COTTER, 2003; HUTKINS, 1993).

On the other hand, growth at high pH values was associated with an increased energetic level that help the bacterial cells maintain their physiological state. At higher pH, metabolic conversions take place at a much faster rate in LAB cells, i.e. decomposing sugars, citric acid and tartaric acid which lead to acetic acid production. These characteristics may represent a benefit for the production of starter cultures used in obtaining fermented products, due to a better control of the quality of the cultures before using them in the food industry (RAULT, 2009).

Growth at different NaCl concentrations

Sodium chloride (NaCl) is the most common curing salt used in fermented products manufacture, acting as a preservative and contributing to the development of the desirable flavor of the foods (CHIKTHIMMAH, 2001). Therefore, we evaluated the resistance of our strains to various NaCl concentrations. The results (Figure 3 and Figure 4) revealed that, while small amounts of NaCl (0.5 to 5%) are well tolerated by most of the strains, higher concentrations of NaCl (>8%) inhibited their growth.

Sodium chloride (NaCl) exhibits both ionic and hyperosmotic stress, the yeasts presenting similar mechanisms of response to these two types of stress. Ion homeostasis and osmotic regulation in yeasts is mediated via two different signaling pathways: calcineurin pathways and the high osmolarity glycerol pathway (HOG), which in fact are interdependent (RODRÍGUEZ-PEÑA, 2010). Physiologically, the activation of these signaling pathways is manifested by shrinking the cells to limit the osmotic pressure followed by the accumulation of counteracting

solutes and restoring the initial physiological state of the cells.

The yeast strains, particularly *I. orientalis* Y-SM3 and *C. parapsilosis* Y-DA1, showed high resistance to NaCl in concentration of 8%. The strains *C. krusei* Y-L3S, *C. krusei* Y-SM3, *C. parapsilosis* Y-DA1 and *H. (O.) polymorpha* CMGB 233 exhibited higher biomass accumulation at 0.5% NaCl compared to the control. This implies that these strains are not only halotolerant, but small concentrations of salts can promote cellular growth (Figure 3). Although *H. (O.) polymorpha* is considered an exception among microorganisms isolated from dairy products since it is not able to utilize lactose or lactic acid for growth or fermentation (two important characteristics of microorganisms from this niche), it is characterized by as a highly stress tolerant yeast (KURTZMAN, 2011). The presence of this species in traditional fermented foods might have two explanations: their ability to utilize secondary metabolites produced by fermentative strains for growth, respectively, their appearance by transfer from the producing environment (MANFRÃO-NETTO, 2019).

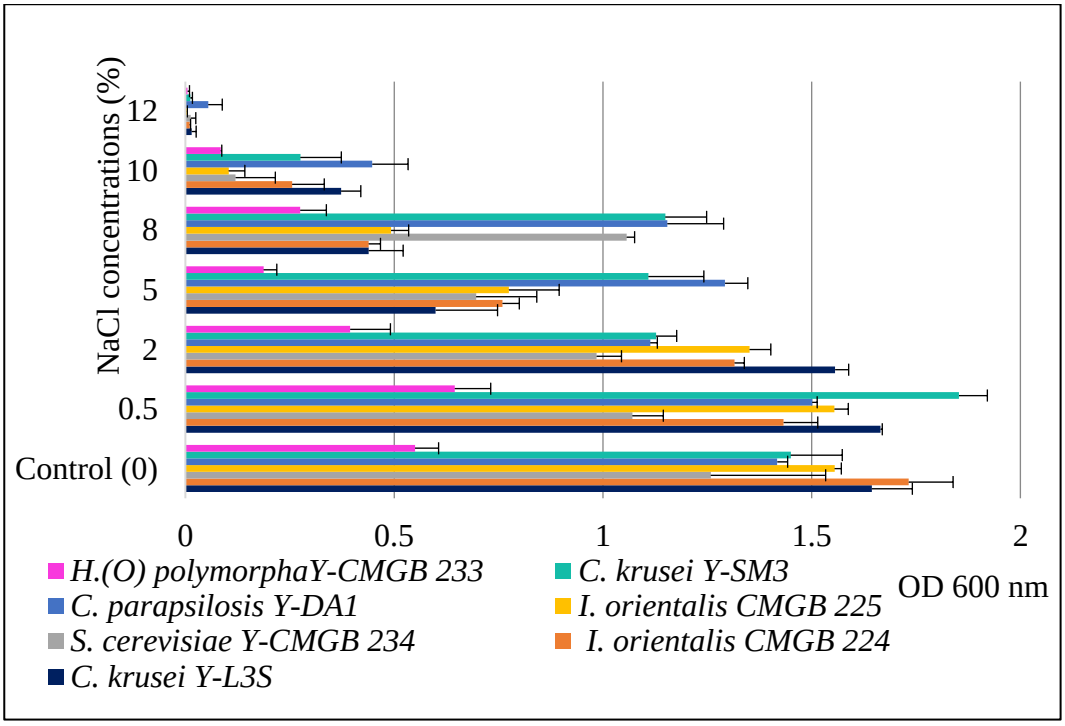

Figure 3. Influence of NaCl concentrations on yeast growth.

The other yeast species (*I. orientalis, C. parapsilosis, C. parapsilosis*) are often characterized as belonging to this ecological niche. Therefore, their resistance to stress conditions induced by high NaCl concentrations is not surprising, recommending them as possible starter cultures for dairy industry.

A similar situation is encountered at LAB strains (Figure 4) that showed an optimal growth at NaCl concentrations of 2%, which also seemed to promote the growth of four strains (LAB-S2b, LAB-SM2, LAB-Bz1 and LAB-Bz6) up to OD 600 nm = 2. Sensitivity of bacterial cultures to salt addition is strongly dependent on bacterial species and strain, and therefore concentrations of NaCl can have either stimulating or inhibitory effect on bacterial metabolic activity. Species belonging to *Lactobacillus* group, *Lactococcus* or even *Streptococcus* genera were found to be able to grow at up to 4% of NaCl (MEDVEĎOVÁ, 2019).

It is interesting that the LAB strains were more affected by the addition of high NaCl concentrations (8 and 10%) compared with the yeasts, most probably due to the changes in cellular metabolism because of its osmotic effect and to the structure of surface proteins of the cells (ARIHARA, 2000).

Growth at various temperatures

Temperature is among the most important factors for manufacturing processes in food industry (MEDVEĎOVÁ, 2019). Temperature variation has a severe effect on microbial growth by affecting the growth rate, the enzymatic activity, cell composition or nutritional requirements or indirectly, by changing the solubility of some molecules and the length of the lag phase and population of microbial species.

Most of the yeasts are mesophilic meaning that they grow best at temperatures between 20 and 30°C. Our strains grew best at 28°C but high OD values were registered also at 37°C and 20°C (Figure 5).

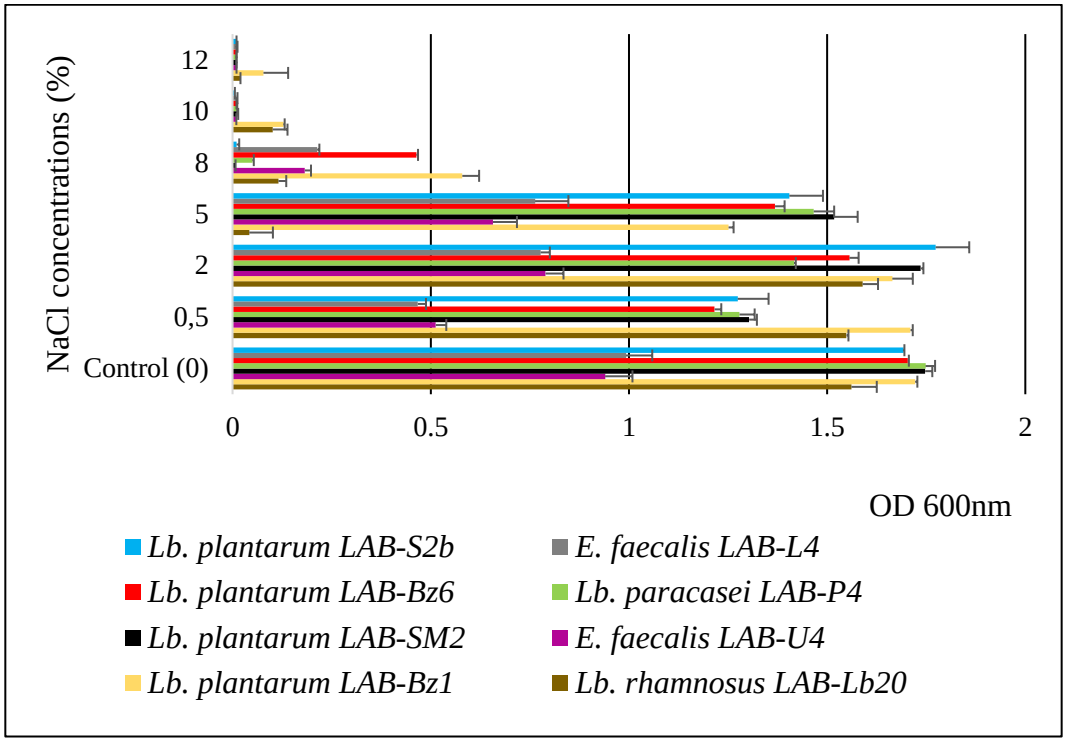

Figure 4. Influence of NaCl concentrations on LAB growth.

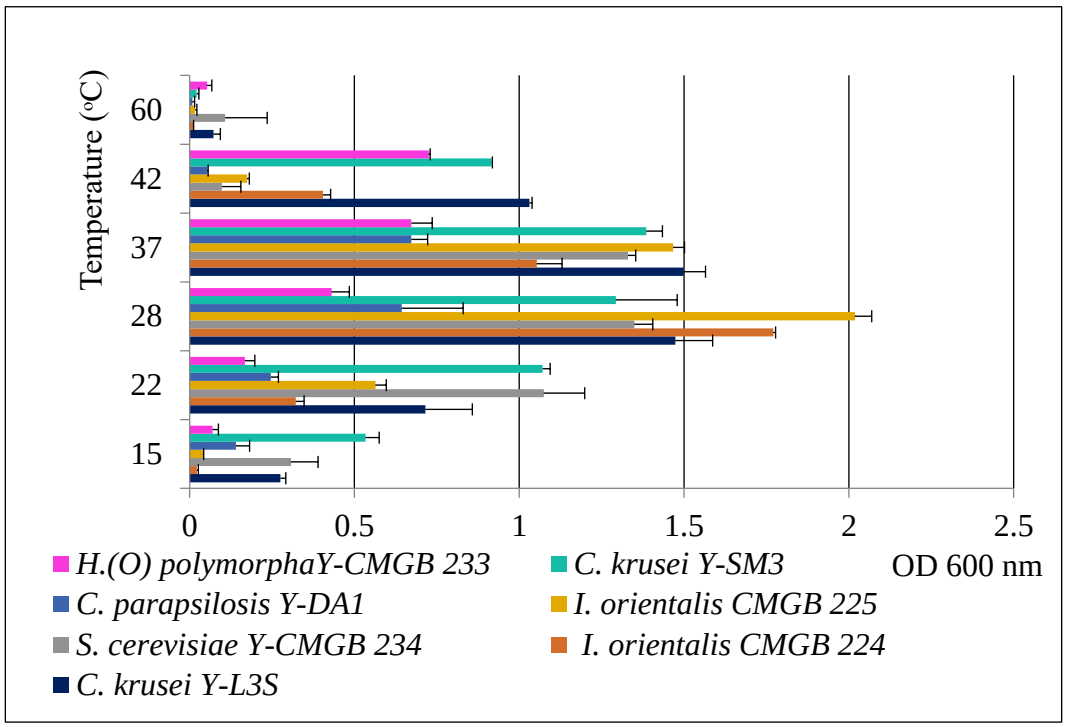

Figure 5. Yeast cultures growth profiles at different temperature values.

The strains *H. (O.) polymorpha CMGB* 233 and *C. krusei* Y-SM3 showed good growth also at 42°C, an important characteristic, this temperature being considered a niche for thermotolerant and thermophilic yeasts. *H. (O.) polymorpha* has unique characteristics being able to grow at temperatures values between 30 to 50°C due to a highly efficient mechanism of heat resistance that involves both the expression of heat-shock protein genes and genes

encoding enzymes from trehalose biosynthesis pathway (ISHCHUK, 2009). Similar physiological behavior was reported for *C. krusei*. This species can accumulate high amount of trehalose and glycerol when exposed to heat stress, which emphasize the biotechnological importance of this species, since both compounds are of special value in food, cosmetic and medical industry (ISHCHUK, 2009). Environments with temperature above 60°C are not suitable for yeasts since this temperature usually affects the stability of the organelles membranes within eukaryote cells.

As shown in Figure 6 the optimal growth temperature for most of the LAB strains analyzed were in the range 28-37°C. On the contrary, for some strains a good growth, up to 1.5 OD 600 nm, was observed at temperatures out of this range: LAB-Lb20 at 42°C; LAB-SM2 and LAB-S2b at 22°C (Figure 6).

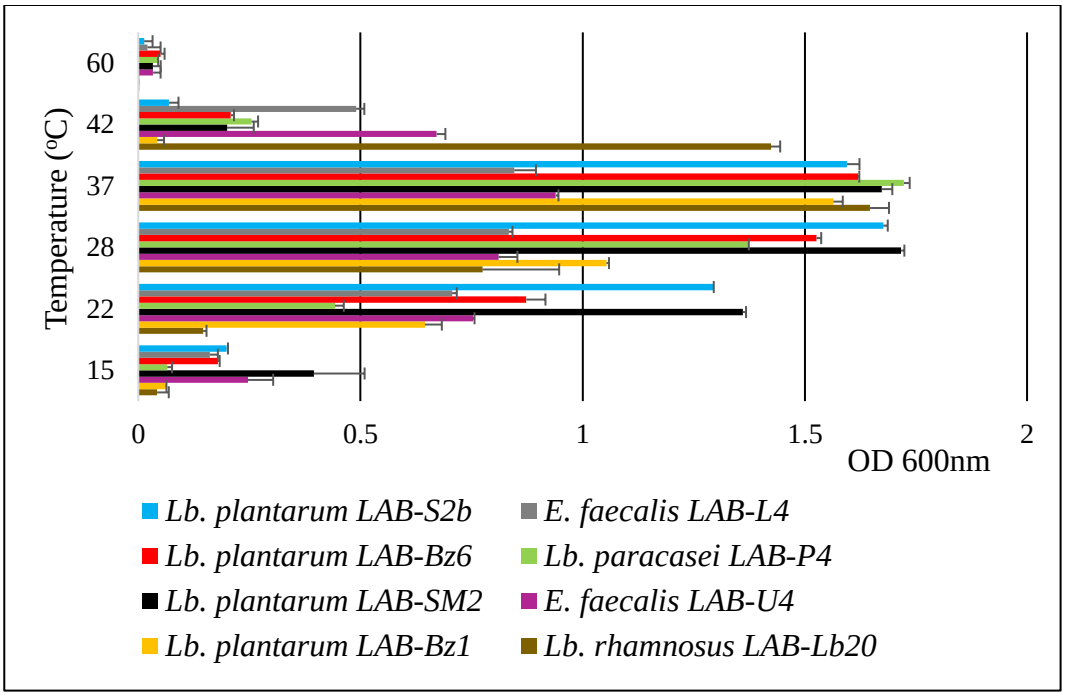

Figure 6. LAB cultures growth profiles at different temperature values.

Although LAB are mesophilic in nature, the temperature range of these microorganisms varies, some species growing well at low or high temperatures. It was shown that low or suboptimal temperatures significantly reduce the dynamic of bacterial populations, as well as the production of lactic acid (slow or absent at temperature less than 20°C) or other inhibitory compounds (AGUILAR, 2010; RUSU, 2016). Nevertheless, other studies reported that LAB strains with growth at low temperature show higher survival rate during long-term conservation (freezing at -70°C) (AHMED, 2006).

Growth is inhibited at temperatures higher than 40°C for most of the LAB species, but there were characterized some *Lactobacillus* species which had a good rate of biomass accumulation at temperatures up to 53°C. These metabolic characteristics are valuable for food industry since the ability of starter cultures to produce organic acids, volatile compounds or carbon dioxide, with a great importance in flavor and texture development, is temperature dependent (ØSTLIE, 2005).

Conclusions

Dairy fermented products play a vital socioeconomic role having a huge rising international market due to its impressive role for human health. Although is a well-developed market, the main players must constantly improve their products in terms of aroma and flavor to meet the increasingly diversified needs of their customers. Traditional fermented foods represent a great source of resources for improving industrial production of dairy products regarding their organoleptic properties (hence the importance of characterizing wild strains from raw materials).

The results of the present study revealed that the growth requirements of yeast and LAB strains are different and complex, showing strain specificity. Although all analyzed strains grew well under their optimal conditions, some of them preferred extreme parameters: acid/very alkaline pH, high temperatures or NaCl concentration.

The yeast strains *I. orientalis* CMGB 225 and *C. krusei* Y-L3S and Y-SM3 along with the LAB strain

Lb. plantarum LAB-SM2 showed high growth when exposed to low pH (3 respectively, 4) and 37°C, representing an important basis for further studies regarding their use in biomedicine. The strains *C. parapsilosis* Y-DA1, *H. (O) polymorpha* CMGB233, *S. cerevisiae* CMGB234, *Lb. plantarum* LAB-S2b, *Lb. plantarum* LAB-SM2, *Lb. plantarum* LAB-Bz1 and *Lb. plantarum* LAB-Bz6 exhibited high resistance to osmotic and pH stress, fact that recommend them for improvement of the biotechnological processes related to the food industry. Among these, *H. (O) polymorpha* CMGB233 proved to be an interesting candidate with valuable applications in industrial processes even though its occurrence in dairy products is mainly due to the transfer from the working environment.

Our results concerning the ability of newly isolated yeast and LAB strains to adapt to stressful environmental conditions helped us to select the best adaptive strains for further studies concerning the production of several metabolites important for food texture or health (exopolysaccharides or biologically active compounds) and for co-cultivation studies in order to develop a mixed starter culture.

Conflict of Interest

The authors have no conflict of interest to declare.

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