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ENHANCEMENT OF BACTERIOCIN PRODUCTION AND ANTIMICROBIAL ACTIVITY OF *PEDIOCOCCUS ACIDILACTICI* HA-6111-2

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The effect of sequential treatments of pressure (50–150 MPa, 10 ºC, 5 min) and temperature (57 ºC, 15 min) on the survival and bacteriocin production of *Pediococcus acidilactici* HA-6111-2 cells in the exponential growth phase was assessed. The growth curves were fitted with the modified Gompertz model, and the estimated maximum specific growth rate was considered to be pressure dependent. A delay in the maximum value of bacteriocin production was registered for more severe pressure conditions, but it was found more notorious for pressure followed by temperature treatments. At lower pressure intensity treatment, regardless of the application order, there was an enhancement of bacteriocin production per cell when compared to the control while maintaining the maximum production value. Bacteriocin production after the treatments can be described by an exponential model.

Keywords: enhancement, bacteriocin production, *Pediococcus acidilactici* HA-6111-2, sequential treatments

Biopreservation of foods could be an effective approach to solve microbial spoilage of food products, to reduce foodborne illness and to address the concern of consumers about the safety of chemical preservatives (Papagianni & Anastasiadou, 2009). Therefore, the search for natural food preservation methods is desirable and has received increasing attention in recent years. Previous reports suggest that bacteriocin-producing bacteria are one of the most promising natural food biopreservatives (Leroy et al., 2003), and lactic acid bacteria (LAB) with bacteriocin-producing abilities, such as pediococci strains, could be used in food manufacturing processes to control the growth of *Listeria monocytogenes* (Dicks et al., 2004) often encountered in fermented foods (Ferreira et al., 2006). Application of bacteriocins as biopreservatives has proven to be effective along the food chain (e.g. veterinary, aquaculture, and agriculture) (Diez-Gonzalez, 2007; Holtsmark et al., 2008; Bakkal et al., 2012).

Non-thermal processes, such as high hydrostatic pressure (HHP), can effectively enhance the safety and shelf life of several food products lacking chemical preservatives without compromising their organoleptic properties and nutritional qualities (Rendueles et al., 2011; Shankar, 2014). HHP has been often described as an economical and technological alternative to thermal processes (Martínez-Rodríguez et al., 2012; Shankar, 2014). In the hurdle concept, simultaneous or sequential techniques that act synergistically are applied to

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control microorganisms in foods. Combining barriers such as biopreservation and HHP could be an attractive way to prevent growth of foodborne pathogens. *P. acidilactici* and their bacteriocins are promising natural food preservatives to control *L. monocytogenes*, but the effects of HHP on *P. acidilactici* are scarcely reported at the present time. Pressures within the range of 400 to 600 MPa are often applied in the food industry. Recently, Castro and co-workers (2015) demonstrated that although *P. acidilactici* HA-6111-2 was inactivated by pressures ≥400 MPa, pressures of ≤200 MPa did not affect bacteriocin production when compared to non-treated cells. Therefore, if used in combination with pressure treatments, bacteriocinogenic LAB cultures might play active role in the control of pathogens when low pressures are used. At ≈300 MPa, processing costs are lower and still can guarantee food safety (Shankar, 2014). As an example, at a certain time and temperature, an increase from 300 to 400 MPa can imply a raise of US $0.013/kg of cheese (Martínez-Rodríguez et al., 2012). Another problem related with LAB bacteriocin commercialization is the low production yields, and as such an enhancement of bacteriocin production should also be addressed. It has been observed that production of bacteriocins is influenced by the bacterial growth phase (Garcia et al., 2016), medium composition (Halami & Chandrashekar, 2005), and culture conditions (Mataragas et al., 2003). In addition, bacteriocin production can be induced by exogenous factors (Neysens & De Vuyst, 2005; Zamfir & Grosu-Tudor, 2009; Vrancken et al., 2011) or exhibit auto-induction (Hakim et al., 2007). Presently, little information is available about the effect of such exogenous factors, including low pressures, on the bacteriocin production. The aim of this study was to evaluate the effect of sequential pressure-temperature treatments in the survival and bacteriocin production of *P. acidilactici* HA-6111-2 cells in the exponential phase of growth.

1. Materials and methods

1.1. Strains and stock cultures

Pediococcus acidilactici HA-6111-2 and *Listeria innocua* 2030c were selected from the *Listeria* Research Center from Escola Superior de Biotecnologia (LRCESB) culture collection. *P. acidilactici* HA-6111-2 cells in the exponential phase of growth (ca. 5 h, 10^7 CFU ml^{-1}) were inoculated at 1% (v/v) in de Man, Rogosa and Sharpe (MRS) broth (Difco Laboratories, Detroit, USA), at pH 6.3.

1.2. Experimental conditions

1.2.1. High hydrostatic pressure treatment. Aliquots (36.0 ml) of *P. acidilactici* HA-6111-2 cell suspensions at the exponential phase were transferred into sterile bottles, aseptically double packed in low permeability polyamide-polyethylene bags (PA/PE-90, Albipack-Packaging Solutions, Águeda, Portugal), and vacuum-sealed to keep out air. The bottles were placed in the high-pressure unit (Unipress Equipment Division, Institute of High Pressure Physics, Warsaw, Poland) and the pressure (HHP) treatments ranged from 50 to 150 MPa (10 ºC, 5 min).

1.2.2. Sequential treatments. For the sequential treatments, aliquots of *P. acidilactici* HA-6111-2 were prepared as described in subsection 1.2.1. A 5 min come up period was considered for the temperature treatment (57 ºC) and further considered as time 0. A lag

period of 2 min was implemented when samples changed from one treatment to the other. Application of pressure followed by temperature was designated as HHP/T, while the other sequence was noted as T/HHP.

1.3. Cell growth and bacteriocin production

P. acidilactici HA-6111-2 treated and non-treated cells at exponential growth phase were cultured in MRS broth (pH 6.3), in duplicate, for 24 h at 37 ºC. The optical density (610 nm) was recorded every hour, and cell enumeration and measurement of bacteriocin activity were performed every three hours. Bacteriocin activity (AU ml^{-1}) was determined according to Van Reenen and co-workers (1998) using *L. innocua* as the target microorganism.

1.4. Mathematical data analysis of growth curves

The modified Gompertz model (Equation 1) was applied to the growth curves in order to determine the maximum specific growth rate (μ_{max}, h^{-1}), the lag time (λ, h), and the maximum reached value (A, dimensionless).

$$\log\left(\frac{N}{N_0}\right) = A\exp\left\{-\exp\left(\frac{\mu_{max}\exp(1)}{A}(\lambda - t)+1\right)\right\} \quad (1)$$

N (CFU ml^{-1}) represents the population density at time t (the index 0 denotes initial values). The mathematical model was fitted to duplicate sets of growth curves data using SPSS® 17.0 for Windows.

2. Results and discussion

2.1. P. acidilactici *HA-6111-2 stability*

According to Figure 1, both low intensity pressures (50–150 MPa, 10 ºC, 5 min) and temperature (57 ºC, 5 min) treatments alone induced ca. 1 log cycle reduction when compared to non-treated cells. No significant ($P<0.05$) differences were registered within the applied pressures due to the fact that low intensities were used. These results are in accordance with previous studies related to pressurization of *P. acidilactici* cells in the late exponential phase (Holtsmark et al., 2008) as significant ($P<0.05$) inactivation only occurred for pressures ≥200 MPa. The same authors stated that there was good correlation between the log CFU ml^{-1} and the pressure level applied for ≥100 MPa, but in the present work such correlation was not observed, cells might be not be sensitive to the pressure range or even to variations of 50 MPa (from 100 to 150 MPa).

The effect of processing conditions related to temperature and pressure at cellular level is often reported as a simultaneous application of both factors, while the present work focused on sequential treatments. When the heat treatment or HHP was introduced seperately, there was ca. 1 log reduction on *P. acidilactici* HA-6111-2 ($P<0.05$). Sequential treatments of HHP/T induced a 0.3–0.6 log CFU ml^{-1} reduction when compared to the ones subjected to pressure treatments alone. Higher reductions (0.4–1.9 log CFU ml^{-1}) were obtained for T/HHP treatments when compared to thermal treatment alone. For pressures ≥100 MPa, the decrease became more pronounced when temperature was applied first.

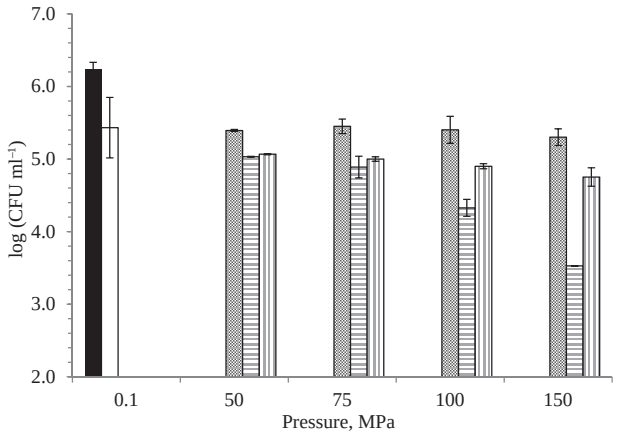

Fig. 1. Survival of exponential phase cells of *P. acidilactici* HA-6111-2 of (■) non-treated (control), and after each treatment: (□) temperature (57 ºC, 15 min) alone; (▨) HHP alone; and sequential conditions (▤ T/HHP; ▥ HHP/T).

2.2. Growth and bacteriocin production

The modified Gompertz model has successfully been applied to growth curves of *P. acidilactici* HA-6111-2 at the exponential growth phase, and the derived key growth values are presented in Table 1. The sequential treatments of pressure and temperature influenced ($P>0.05$) the μ_{max} at higher pressures (≥75 MPa), and the effect was more enhanced for temperature followed by pressure (T/HHP). For both sequences, with the exception of HHP(50)/T, the estimated μ_{max} was considered to be pressure dependent: the experimental data were fitted into a linear model, with a good correlation (HHP/T: $\mu_{max} = -8.4\times10^{-4}$ P+0.243; R=0.985; T/HPP: $\mu_{max} = -1.44\times10^{-3}$ P+0.448; R=0.864). Contrary to the conclusions of Castro and co-workers (2015), for pressure treated (200–500 MPa) *P. acidilactici* HA-6111-2 in the stationary phase of growth no correlation was found between A and pressure. The order of the sequential treatments was relevant; higher survival values were observed for cells firstly submitted to pressure (HHP/T), and mainly for higher pressure values. Under the applied experimental conditions, no reliable estimates regarding the λ were obtained due to limited data.

Table 1. Parameter estimation (μ_{max} and A) obtained from the modified Gompertz model (Equation 1) for growth curves of *P. acidilactici* under the applied sequential treatment conditions

Pressure (MPa)	μ_{max} (h^{-1})	A	R^2
Control (0.1)	0.432±0.063^{a}	2.916±0.057^{a}	0.979
HHP(50)/T	0.443±0.071^{a}	2.895±0.027^{a}	0.975
HHP(75)/T	0.310±0.016^{b}	3.791±0.064^{b}	0.995
HHP(100)/T	0.320±0.021^{b,c}	4.248±0.051^{c}	0.988
HHP(150)/T	0.371±0.041^{a,c}	3.894±0.067^{b}	0.984
T/HHP(50)	0.415±0.032^{a}	3.117±0.109^{d,e}	0.994
T/HHP(75)	0.299±0.006^{b}	3.177±0.124^{d}	0.999
T/HHP(100)	0.287±0.013^{b}	2.982±0.057^{a,e}	0.996
T/HHP(150)	0.251±0.021^{d}	3.173±0.031^{d}	0.979

Values with different letters in the same column are significantly different ($P<0.05$).

Cells presented a maximum value of bacteriocin production of 6400 AU ml^{-1} between 18–21 h, followed by a decrease down to 1600 AU ml^{-1} (Fig. 2). Similar results were previously reported and attributed to the reduction of pH due to production of lactic acid as the LAB cells grow, adsorption to cells, or bacteriocin aggregation (Aansen et al., 2000), and/or to the production of proteolytic enzymes by the cells in stationary growth phase (Ikram-Ul & Mukhtar, 2006). The antimicrobial activity of bacteriocin produced by *P. acidilactici* HA-6111-2 after sequential treatments is plotted in Figure 2 (A and B). The sequential treatments of T/HHP (Fig. 2B) induced a higher decrease in the production of bacteriocin than the HHP/T (Fig. 2A). As can be seen in Figure 2A, all applied sequential treatments HHP/T revealed a maximum bacteriocin production of 6400 AU ml^{-1}, but with a delay of ca. 3 and 6 hours for HHP(75)/T and HHP(100 and 150)/T, respectively, when compared to both control and the less intense treatment (HHP(50)/T). It is possible that cells become injured in such a way that they need to recover before starting bacteriocin production. The sequence of the treatments was important on the above-mentioned delay, and more notorious for pressure followed by temperature (Fig. 2).

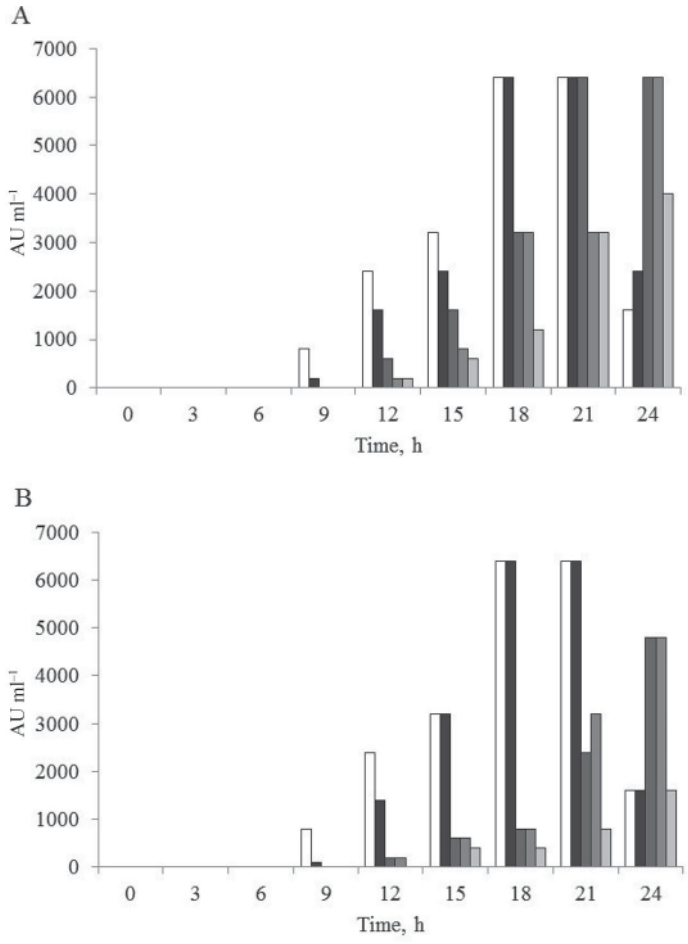

Fig. 2. Antimicrobial activity of bacteriocin produced by *P. acidilactici* HA-6111-2 cells after sequential treatments: (A) HHP/T; (B) T/HHP. The legend of both graphics is □: control; ■: 50 MPa; ■: 75 MPa; ■: 100 MPa; ■: 150 MPa

From the sequential treatments of T/HHP, the lowest pressure intense treatment (T/HHP(50)) was the only one that presented a similar bacteriocin production curve when compared to the control: an increase of bacteriocin production up to a maximum value (6400 AU ml^{-1}), followed by a declination (Fig. 2B). The other sequential T/HHP treatments reached a lower maximum value of bacteriocin production (4800 and 1600 AU ml^{-1}), without a declination. It is possible that the antimicrobial activity corresponding to this treatment would increase after 24 h, but it seemed unlikely that it would reach a maximum value of 6400 AU ml^{-1}. After a sub-lethal heat treatment, cells could become more vulnerable and their baroresistance decrease (Rendueles et al., 2011), despite the fact that the cell growth seemed to have recovered from the treatment.

When bacteriocin production per cell (AU CFU^{-1}) was plotted over time (Fig. 3), a delay was again observed for pressures of ≥75 MPa. This effect was due to the sequential treatments applied rather than the differences in initial cells counts alone. It was also noticed that 50 MPa treatment, regardless of the application order, induced a higher bacteriocin production per cell (ca. 2.3-fold) than the control, while maintaining the maximum production value (6400 AU ml^{-1}), without altering the maximum specific growth rate and the maximum reached value (Table 1). This increase in bacteriocin production may occur as a response to exposure to sub-lethal pressure conditions, as it has been previously reported that bacteriocin production is stimulated by various stress conditions (Neysens & De Vuyst, 2005; Zamfir & Grosu-Tudor, 2009; Vrancken et al., 2011). Nevertheless, the enhancement of the bacteriocin production due to cells exposure to low pressure treatments needs to be further investigated. To our knowledge, this effect has never been reported before.

Bacteriocin production can be also modelled that may further elucidate underlying metabolic regulatory mechanisms and lead to a faster and easier inoculum build-up optimization necessary for ultimate up-scaling. Bacteriocin production increased exponentially ($AU\ CFU^{-1}=A\times e^{(B\times t)}$) with a good correlation (R≥0.920). As it can be seen in Table 2, the order of application of the treatments affected differently the two coefficients: coefficient A is higher for sequential treatments where pressure was firstly applied; coefficient B was higher when application of temperature was followed by pressure. Regardless the sequence of the treatment, coefficient A presented the highest value for 75 MPa.

Table 2. Parameter estimation for bacteriocin production of *P. acidilactici* HA-6111-2 cells

Pressure (MPa)	$A\times10^{-2}$	B	R^2
Control (0.1)	6.7±2.5[a]	0.215±0.022[a]	0.987
HHP(50)/T	7.0±4.4[a]	0.255±0.036[a]	0.981
HHP(75)/T	13.1±4.3[b]	0.162±0.055[b]	0.936
HHP(100)/T	8.1±3.0[a]	0.153±0.016[b]	0.978
HHP(150)/T	1.2±1.2[c]	0.279±0.052[a]	0.966
T/HHP(50)	1.4±0.6[c]	0.345±0.024[c]	0.997
T/HHP(75)	9.6±7.2[b]	0.160±0.034[b]	0.926
T/HHP(100)	4.7±4.9[a]	0.190±0.046[b]	0.920
T/HHP(150)	0.6±0.2[d]	0.234±0.016[a]	0.997

Values with different letters in the same column are significantly different ($P<0.05$).

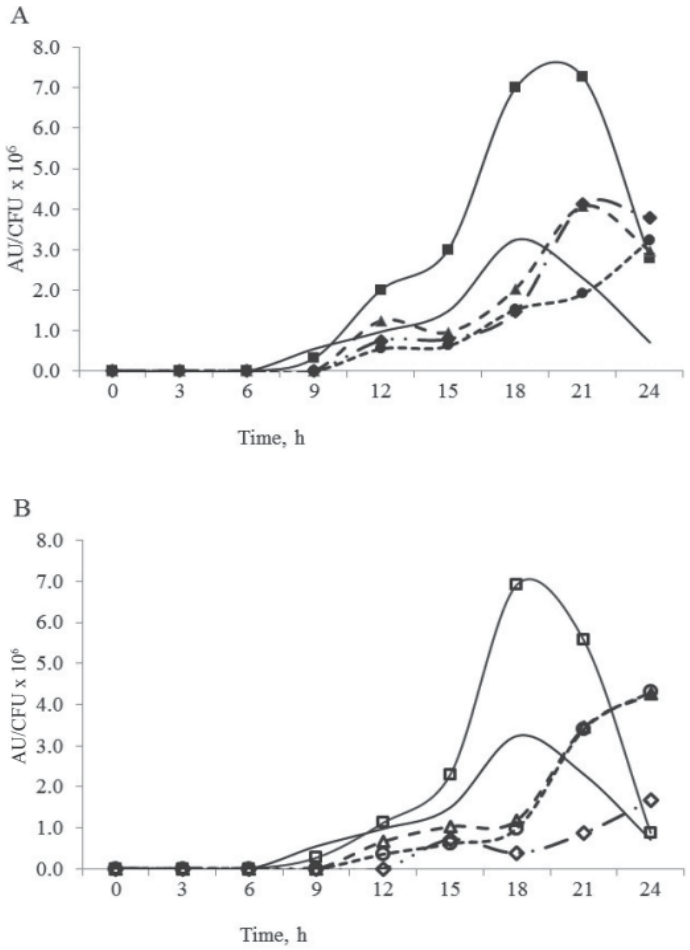

Fig. 3. Antimicrobial activity of bacteriocin per cell produced by *P. acidilactici* HA-6111-2 after sequential treatments. (A) HHP/T (full line – control; ■: 50 MPa; ▲: 75 MPa; ●: 100 MPa; ◆: 150 MPa); (B) T/HHP (full line – control; □: 50 MPa; △: 75 MPa; ○: 100 MPa; ◇: 150 MPa).

3. Conclusions

P. acidilactici HA-6111-2 cells revealed to be more sensitive when temperature was applied before pressure, particularly for ≥100 MPa. The growth curves of treated cells were described by the modified Gompertz model, and the maximum specific growth rate was pressure dependent. The sequential application of temperature followed by pressure treatments was detrimental to cell growth, while the bacteriocin production per cell was enhanced when low pressure intensities were applied, despite the application order. This effect was attributed to the applied sub-lethal pressure conditions, similar to other stress conditions. The bacteriocin production was described by an exponential model.

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References

Aansen, I.M., Møretrø, T., Katla, T., Axelsson, L. & Storrø, I. (2000): Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687. *Appl. Microbiol. Biot., 53*, 59–66.

Bakkal, S., Robinson, S.M. & Riley, M.A. (2012): Bacteriocins of aquatic microorganisms and their potential applications in the seafood industry. -in: Carvalho, E.D. (Ed.), *Health and environment in aquaculture*. INTECH Open Access Publisher, Rijeka, Croatia. pp. 304–328.

Castro, S.M., Kolomeytseva, M., Casquete, R., Silva, J., Saraiva, J.A. & Teixeira, P. (2015): Effect of high pressure on growth and bacteriocin production of *Pediococcus acidilactici* HA-6111-2. *High Pressure Res., 35*, 405–418.

Dicks, L.M., Mellett, F.D. & Hoffman, L.C. (2004): Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich meat salami. *Meat Sci., 66*, 703–708.

Diez-Gonzalez, F. (2007): Applications of bacteriocins in livestock. *Current Issues in Intestinal Microbiology (CIIM), 8*, 15–23.

Ferreira, V., Barbosa, J., Vendeiro, S., Mota, A., Silva. F., Monteiro, M.J., Hogg, T., Gibbs, P. & Teixeira, P. (2006): Chemical and microbiological characterization of alheira: A typical Portuguese fermented sausage with particular reference to factors relating to food safety. *Meat Sci., 73*, 570–575.

Garcia, J.M., Castro, S.M., Casquete, R., Silva, J., Queirós, R., Saraiva, J.A. & Teixeira, P. (2016): Mild pressure treatments applied to *Pediococcus acidilactici* HA-6111-2 at exponential growth phase. -in: Padilla, T. (Ed.), *Bacteriocins: Production, applications and safety*. Nova Science Publishers, Inc. New York, pp. 137–152.

Hakim, G., Noreddine, B., Dalié, D.D., Frank, D. & Philippe, T. (2007): Comparison of the performances of different fermentation strategies on cell growth and bacteriocin production by *Lactobacillus curvatus* CWBI-B28. *J. Sci. Food Agr., 87*, 541–549.

Halami, P.M. & Chandrashekar, A. (2005): Enhanced production of pediocin C20 by a native strain of *Pediococcus acidilactici* C20 in an optimized food-grade medium. *Process Biochem., 40*, 1835–1840.

Holtsmark, I., Eijsink, V.G. & Brurberg, M.B. (2008): Bacteriocins from plant pathogenic bacteria. *FEMS Microbiol. Lett., 280*, 1–7.

Ikram-Ul, H. & Mukhtar, H. (2006): Biosynthesis of protease from *Lactobacillus paracasei*: kinetic analysis of fermentation parameters. *Indian J. Biochem. Bio., 43*, 377–381.

Leroy, F., Foulquie Moreno, M.R. & De Vuyst, L. (2003): *Enterococcus faecium* RZS C5, an interesting bacteriocin producer to be used as a co-culture in food fermentation. *Int. J. Food Microbiol., 88*, 235–240.

Martínez-Rodríguez, Y., Acosta-Muñiz, C., Olivas, G.I., Guerrero-Beltrán, J., Rodrigo-Aliaga, D. & Sepúlveda, D.R. (2012): High hydrostatic pressure processing of cheese. *Compr. Rev. Food Sci. F., 11*, 399–416.

Mataragas, M., Metaxopoulos, J., Galiotou, M. & Drosinos, E.H. (2003): Influence of pH and temperature on growth and bacteriocin production by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442. *Meat Sci.*, *64*, 265–271.

Neysens, P. & De Vuyst, L. (2005): Carbon dioxide stimulates the production of amylovorin L by *Lactobacillus amylovorus* DCE 471, while enhanced aeration causes biphasic kinetics of growth and bacteriocin production. *Int. J. Food Microbiol.*, *105*, 191–202.

Papagianni, M. & Anastasiadou, S. (2009): Pediocins: The bacteriocins of *Pediococci*. Sources, production, properties and applications. *Microb. Cell Fact., 8*, 1–16.

Rendueles, E., Omer, M.K., Alvseike, O., Alonso-Calleja, C., Capita, R. & Prieto, M. (2011): Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT - Food Sci. Technol., 44*, 1251–1260.

Shankar, R. (2014): High pressure processing – Changes in quality characteristic of various food material processed under high pressure technology. *Int. J. Innov. Sci. Res. (IJISR), 3*, 168–186.

Van Reenen, C.A., Dicks, L.M. & Chikindas, M.L. (1998): Isolation, purification and partial characterization of plantaricin 423, a bacteriocin produced by *Lactobacillus plantarum*. *J. Appl. Microbiol., 84*, 1131–1137.

Vrancken, G., De Vuyst, L., Rimaux, T., Allemeersch, J. & Weckx, S. (2011): Adaptation of *Lactobacillus plantarum* IMDO 130201, a wheat sourdough isolate, to growth in wheat sourdough simulation medium at different pH values through differential gene expression. *Appl. Environ. Microb.*, *77*, 3406–3412.

Zamfir, M. & Grosu-Tudor, S. (2009): Impact of stress conditions on the growth of *Lactobacillus acidophilus* IBB 801 and production of acidophilin 801. *J. Gen. Appl. Microbiol.*, *55*, 277–282.