International Food Research Journal 25(5): 2131-2137 (October 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

The efficacy of bacteriocin-containing cell-free supernatant from *Lactobacillus plantarum* Cys5-4 to control pathogenic bacteria growth in artisanal beverages

*Tenea, G.N. and Barrigas, A.

Department of Biotechnology, Faculty of Engineering in Agricultural and Environmental Sciences, The Technical University of the North, Av. 17 de Julio s-21 y José María Córdova. Postcode: 199, Ibarra, Barrio El Olivo, Ecuador

Article history

Received: 11 July 2017
Received in revised form: 27 September 2017
Accepted: 13 October 2017

Keywords

Antimicrobial activity
Pathogen control
Bacteriocin
Preservation
Fresh beverages

Abstract

The increasing interest on consuming fresh, non-processed healthy food and beverages expand the research on finding new alternatives for natural preservation. Antimicrobial substances produced by lactic acid bacteria proved to be safer for health making them of great interest to be investigated. In the current study, the bacteriocin-producing *Lactobacillus plantarum* Cys5-4 strain has been targeted for its preservative potential in artisanal beverages. The maximum production was registered at the stationary phase and increased when glycerol (5%) was added to bacterial culture medium. Agar-well diffusion assay indicated that cell-free supernatant (CFS) containing active substances and precipitated bacteriocin (PP) displayed a broad range of antimicrobial activity towards several indicator strains. When CFS Cys5-4 was applied to orange juice at the room temperature, about two-fold reduction in viable cell of *E. coli* from 6.0 log CFU/ml at the inoculation time to 3.03 log CFU/ml on Day 5 was registered. However, a decrease of *Salmonella* was registered on Day 1 (4.24 log CFU/ml) and maintained in the same range during storage period. Similarly, a gradual reduction of viable cells towards both pathogens tested was registered in chicha beverage stored at 4°C. These results revealed the high potential of bacteriocin Cys5-4 to control pathogenic bacteria growth in natural beverages thus would be effective as overall good manufacturing practice of those products.

Introduction

The increasing consumer demand for fresh, non-processed and healthy foods has expanded in the last decade; however the susceptibility to contamination with spoilage microorganisms remains elevated. Improper manipulation, the usage of contaminated water, inappropriate storage conditions as well as inefficient methods for long term conservation are considered the main causes of pathogenesis (Sperber, 2009). Nowadays, the tendency of replacing traditional methods for conservation by natural ones open the possibility to explore the use of microorganisms or their antimicrobial substances (Cleveland *et al.*, 2001; Delvez-Broughton, 2005; Deegan *et al*; 2006; Ishiyama *et al*., 2008). In the particularly case of fresh juices, the conservation is based mainly on thermal processing and consequently reducing the product quality and freshness (Todorov, 2009; Nath *et al.*, 2014). Moreover, the chemical preservatives used in preservation had adverse effects on the consumer health (Nath *et al*., 2014). Thus, with the expectation of achieving the food safety standards, traditional means of controlling the risk of microbial putrefaction are being retrieved by innovative technologies including biological systems such as the use of lactic acid bacteria and / or its antibacterial components (i.e. organic acids, diacetyl, bacteriocins) (Cleveland *et al*., 2001; Deegan *et al.*, 2006; Parada *et al.*, 2007; Rico *et al*., 2007; Hartmann *et al*., 2011) without drawbacks in alteration of organoleptic or nutritional properties of food (Amin *et al*., 2012). For decades, lactic acid bacteria were used in food fermentation and / or preservation (Winkelstroter *et al.*, 2011). Bacteriocins produced by lactic acid bacteria are considered generally regarded as safe (GRAS) (Messauodi *et al*., 2013; Xin *et al*., 2015) due to their low molecular weight bacteriocins and binds to the cell surface receptors of indicator strains. The molecular mechanism includes formation of pores, cellular DNA degradation, and disruption by specific cleavage of 16S rDNA and inhibition of peptidoglycan synthesis (Todorov, 2009). The greatest contribution of these microorganisms is the preservation of the nutritional quality of the raw material through the increase of the shelf-life and

*Corresponding author.
Email: *gntenea@utn.edu.ec, gtenea@hotmail.com*

the inhibition of some pathogen growth (Nath *et al.*, 2014). Nonetheless, Nisin of *Lactococcus lactis* remains the only commercial bacteriocin used in the market and many other are under study (Delves-Broughton, 2005; Taheri *et al*., 2012; Biscola *et al*., 2013).

In Ecuador, the consumption of fresh fruits and traditional fermented beverages is elevated; consequently, preservation using natural methods is necessary. The mild climate permits the production of tropical fruits for fresh juices, but at the same time, favors the proliferation of pathogenic microorganisms. The fresh artisanal beverages are additives free and usually the method of preparation is not disclosed by the provider. For example, chicha beverage, consumed in massive quantities, is obtained from the fermentation of seven varieties of corn; all after a drying, grinding and boiling process. Similarly, the fresh orange juice is produced in larger quantities and sold in the local market as convenient and cheaper choice. Nonetheless, the contamination with *Salmonella enterica* serovar Thyphimurium, *Escherichia coli* 0157: H7 and / or *Shigella*, is foreseeable (Tenea and Yepez, 2016).

Previously, we identified and selected several native lactic acid bacteria (LAB) showing probiotic and antimicrobial potential (Benavidez *et al.*, 2016). More recently, the antimicrobial components of some LAB strains isolated tropical wild-type fruits of Amazon were characterized. In the effort to find a new antimicrobial agent, we focused attention on *Lactobacillus plantarum* assigned Cys5-4 strain (Garzón *et al.,*2017). In the present study, we evaluated the inhibitory spectrum of bacteriocin producing Cys5-4 strain in two forms, cell-free supernatant (CFS) containing active substances and precipitated peptides (PP), the optimum production along with the effectiveness to control the growth of pathogenic microorganisms in artisanal fresh orange and chicha beverage.

Materials and Methods

Bacterial strains and culture conditions

Lactobacillus plantarum Cys5-4 isolated from tropical wild-type fruits of Malus sp. (Sucumbíos Provence of Ecuador) was grown in MRS broth medium (Merck) at 32°C, under anaerobic conditions. The strain was registered at GenBank with the assignation number: KY041686. As reference strain in all experiments the *L. plantarum* ATCC 8014TM was used. The microorganisms were preserved by deep-freezing in glycerol solution before use.

Growth curve and bacteriocin production

Fresh culture of *Lactobacillus plantarum* Cys5-4 obtained after 18 hours of incubation was used to inoculate (2%, v/v) MRS broth. The obtained culture (100 ml) was incubated at 32°C in static conditions. The optical density (OD_{605} nm) was measured at intervals of 3 hours for 30 hours. The bacteriocin production was monitored at different intervals during bacterial growth by measuring the inhibition zone produced by cell-free supernatant (CFS) using agar well diffusion assay (Benavidez *et al.*, 2016). Briefly, CFS collected by centrifugation at 13,000 x g for 20 minutes (4°C) was recovered and filtered using 0.22 µm porosity syringe filter. The indicator strain (100 µl) grown in broth medium (7 log CFU/ml) was mixed with 3.5 ml of soft MRS agar (0.75%), overlaid on the nutrient agar plates and incubated at 37°C for 2 hours. The CFS (100 µl) was transferred onto the wells (6 mm) on overlaid agar, incubated at 37°C and subsequently examined for inhibition zones at different intervals of time (24-48 hours). As indicator the *E. coli* ATCC 25922 was used.

Effect of medium composition on bacteriocin production

The MRS medium was modified as follow: 1) MRS broth supplemented with sucrose (2%); 2) MRS broth supplemented with KH_2PO_4 (2%); 3) MRS broth supplemented with 5% glucose; 4) MRS broth supplemented with 1% and 5% Tween 20; 5) MRS broth supplemented with 5% glycerol. As control MRS broth without nutrients has been used. The Cys5-4 has been inoculated individually in each of the MRS medium combination for 24 hours and the CFS obtained as mentioned above was used on agar-well diffusion assay. As reference strains both *E. coli* ATCC 25922 and *Salmonella enterica* ATCC 51741 were used. Each experiment was performed in triplicates starting from individual bacterial culture. The bacteriocin titer was expressed as arbitrary units per ml (AU/ml). One arbitrary unit was defined as the highest dilution showing about 2 mm of inhibition zone on the indicator lawn (Todorov, 2013).

Determination of antimicrobial spectrum

Antimicrobial activity of both CFS and PP Cys5-4 form was performed using the agar-well diffusion method (Benavidez *et al*., 2016). Two sets of reference indicator strains were used: 1) Gram-positive: *Lactobacillus acidophilus* ATCC 4356 *Streptococcus thermophiles* ATCC 19258, *Bifidobacterium* breves ATCC 15700 grown in MRS broth medium; 2) Gram-negative: *E. coli* ATCC 25922, *Salmonella enterica* subsp. *enterica* ATCC 51741 (Kauffmann and Edwards Le Minor and Popoff), and *Shigella*

sonnei ATCC 23931 as well as *E. coli* UTN Ec1, *Enterobacter aerogenes* UTN Ea1, *Salmonella* UTN Sm2 and *Shigella* UTN Shg1 isolated from local fresh cheese. To obtain precipitated peptides, the CFS was treated with 60% ammonium sulfate, incubated overnight at 4°C and centrifuged at 8000 x g for 30 minutes. The PPs were recovered in ammonium acetate 25 mM and stored at (-) 20 before use. The experiments were run in triplicates the mean values of the inhibition zones were estimated.

Effect of bacteriocin-containing Cys5-4 on the viability of pathogenic bacteria on artisanal beverages

The effect of CFS containing Cys5-4 bacteriocin in the pathogen growth was evaluated on fresh orange and fermented chicha juice, procured from ambulatory local vender. Different sets of experiments were performed: 1) Investigation of pathogen presence in orange juice. Briefly, the juice was inoculated in SS (*Shigella-Salmonella,* Difco) and eosin methylene blue (Difco) agar plates, incubated for 48 hours at 37-40°C for detection of *Shigella, Salmonella* and *E. coli*. Subsequently, the juice was heat treated for 10 minutes at 100°C to eliminate the contaminants. 2) Investigation of antimicrobial effect of Cys5-4. *E. coli* ATCC 25922 and *Salmonella enterica* subsp. *enterica* ATCC 51741 were artificially inoculated in juice samples at the concentration of 6 log CFU/ml. The experiment was carried out in sterile bottles (A, B, C, D and E) containing 50 ml of orange juice as following: Bottle A (control) containing juice without any pathogen and preservative added. Bottle B: juice with the indicator *E. coli* with Cys5-4 CFS added at the final concentration of 128AU/ml. Bottle C: containing the indicator *Salmonella enterica* in which the same concentration of Cys5-4 CFS was inoculated. Bottle D: juice with the indicator *E. coli* in which CFS was replaced with sterile water. Bottle E: juice with the indicator *Salmonella* in which CFS was replaced with sterile water. Similar experiment was applied for chicha juice. All bottles were incubated for 5 days at room temperature in case of orange juice and 4°C for chicha and the effect of the CFS containing bacteriocin Cys5-4 on the pathogen viability was monitored at each 24 hours using agar plate assay method (Pratush *et al.*, 2012). The samples were mixed, homogenized and the pH was daily measured. The experiments were performed in triplicates starting with different batches of beverages.

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance followed by Duncan's test and the results were considered statistical significant at the $P < 0.05$ level (SPSS version 10.0.6, USA and Excel).

Results and Discussion

Growth curve and bacteriocin production

The bacteria reached stationary phase after 24 hours of incubation. According with the registered inhibition zones the production of Cys5-4 bacteriocin was detected at 3 hours of bacterial growth suggesting that the released peptides are primary metabolites (Figure 1). The maximum activity was registered at 24 hours and maintained up to 30 hours indicating that the bacteria Cys5-4 must be grown for at least 24 hours to get optimum activity. Similarly, when studying bacteriocin of *L. brevis* FPTLB3, the maximum production was detected at the end of logarithmic phase of the bacterial growth (Banerjee *et al.*, 2013). If the initial pH of Cys5-4 was 6.0, after 24 hours of incubation declined at 3.8-4.0, which correlates with the optimum production of bacteriocin. Similar trends on the bacteriocin production were early reported (Zamfir *et al*., 2000; de Arauz *et al*., 2009).

Effect of different medium composition on the bacteriocin production

The bacteriocin production was maintained stable when increasing the concentration of sugars (2%) and Tween 20 (1 and 5%) while soluble salts decreases with 50% the production (Table 1). Early studies showed that several nutrients controlled the bacteriocin production (Keren *et al.*, 2004). For example, sugars negatively regulated the bacteriocin production of *Lactobacillus sakei* ST22CH (Todorov *et al*., 2013). Previous studies indicated the role of glycerol with sugars in cellular growth of some *Lactobacillus* species (Teusink *et al.*, 2009) but no study demonstrated a direct link between glycerol and an increase in the antimicrobial compounds. In other study, glycerol had negatively regulated the plantacirin ST31 production (Todorov *et al.*, 2000). In this study, as shown in the Table 2, glycerol (5%) was the only component that stimulated the overall bacteriocin production and we used this medium for further experiments.

The spectrum of antimicrobial activity of bacteriocin-produced by Cys5-4 strain

In general, bioactive substances of lactic acid bacteria suppress the growth of species from the same genus and some pathogenic microorganisms (Todorov, 2009). This is an important property

Table 1. Effect of medium composition on bacteriocin Cys5-4 production

Component Concentration	Bacteriocin activity (AU/ml)	
	E. coli ATCC 25922	*Salmonella enterica* ATCC 51741
MRS + Tween 20 (1%)	6400	6400
MRS + Tween 20 (5%)	6400	6400
MRS + Glycerol (5%)	12800	12800
MRS + Glucose (2%)	6400	6400
MRS + Sucrose (2%)	6400	6400
MRS + KH_2PO_4 (2%)	3200	3200
MRS	6400	6400

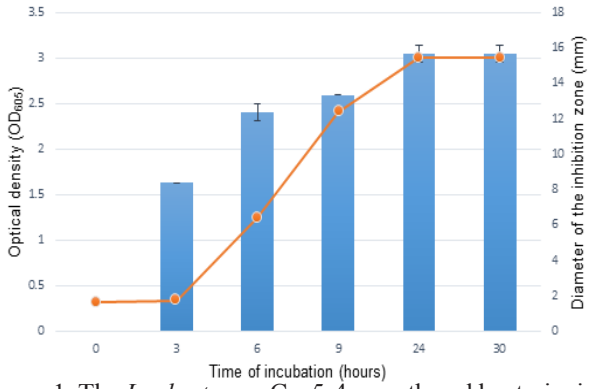

Figure 1. The *L. plantarum* Cys5-4 growth and bacteriocin production at different time of incubation in MRS broth. The error bars indicate standard error of the mean of three replicates.

of a probiotic strain allowing its colonization and competitiveness edge over other bacteria found in the same niches (Todorov *et al*., 2013). In this study, the antimicrobial activity of bacteriocin producing *L. plantarum* Cys5-4 strain was evaluated against two sets of indicator strains: Gram-positive and Gram-negative. Results from agar well assay indicate that the CFS Cys5-4 containing the bacteriocin had a broad range spectrum of activity (Table 2). No significant difference on inhibitory activity, nor between CFS and PP was observed towards Gram-positive indicator strains. A significant difference ($P < 0.05$) on the inhibitory activity towards Gram-negative bacteria tested is shown as well as a significant difference in inhibitory activity between CFS form and PP counterpart was detected, suggesting that the exerted killing action of CFS might be related to the presence of acids. Early research suggested that the use of cell-free supernatant rather than purified bacteriocin might be an advantage if further using in preservation (Hartmann *et al*., 2011; Arena *et al*., 2016). Direct application of bacteriocin in food matrix might conduct to a reduction or loss of activity over time as effect of enzymatic degradation. The results showed that CFS Cys5-4 inhibits all target Gram-negative

Table 2. Spectrum of antimicrobial activity of bacteriocin Cys5-4.

Indicator Strains	Diameter of the inhibition zone (mm)	
	CFS	PP
Gram-positive		
***Bifidobacterium breves* ATCC 15700**	12.66 ± 1.25	11.66 ± 0.47
***Streptococcus thermophiles* ATCC19258**	12.33 ± 0.94	11.33 ± 0.82
***L. acidophilus* ATCC 4356**	12.00 ± 0.00	11.33 ± 0.94
Gram-negative		
***E. coli* ATCC 25922**	21.66 ± 1.24^{Aa}	15.33 ± 0.82^{Aa}
***Shigella sonnei* ATCC 25931**	19.33 ± 0.94^{Bb}	13.66 ± 1.25^{Bb}
***Enterobacter aerogenes* UTN Ea1**	16.33 ± 1.25^{Cc}	12.33 ± 0.81^{Cc}
***Salmonella enterica* subsp. *enterica* ATCC 51741**	15.00 ± 0.82^{Dd}	12.00 ± 0.00^{Cc}
***E. coli* UTN Ec1**	14.66 ± 0.94^{Ee}	12.33 ± 0.94^{Cc}
***Salmonella* UTN Sm2**	12.66 ± 0.94^{F}	11.33 ± 0.47^{D}
***Shigella* UTN Shg1**	12.66 ± 0.94^{F}	11.00 ± 0.00^{D}

* Data are means ± standard error. Mean in the same column that are followed by different capital letters are significantly different ($p < 0.05$); Small letters in the same row indicate that differences between CFS and PP are significant (Duncan's test). CFS: cell-free supernatant, PP-precipitated peptides.

strains and qualified for further application *ex vitro* towards different food matrix for shelf-life extension.

Efficacy of cell-free supernatant containing bacteriocin Cys5-4 to control the E. coli *and* Salmonella *growth in artisanal juices*

Considering that artisanal drinks are highly consumed and in most cases sold out without previous verification, at the initial point of experiments we analyzed the presence of contaminants in both beverages, orange juice and chicha. The microbiological analysis showed that both juice samples contained *E. coli* and *Salmonella*, indicating that any normative enforced for fresh fruits and vegetables (NTS INEN 2337, 2008) was considered. However, to overcome this problem the efficiency of bacteriocin Cys5-4 CFS to control the growth of pathogens in both beverages was evaluated. The results showed that the application of CFS Cys5-4 in orange juice reduced the viable pathogen cells when compared with untreated control (Figure 2). The *E. coli* cell density decreased from 6.0 log CFU/ml at the inoculation point at 5.7 log CFU/ml on Day 1 when reduced progressively at 3.03 log CFU/ml on Day 5. In the control samples (only pathogen added), the cell density reduced at 5.29 log CFU/ ml on Day 2 followed by an increase at 6.66 on Day 5, suggesting that the pathogen found the appropriate environment to proliferate even if acidic conditions. Those data

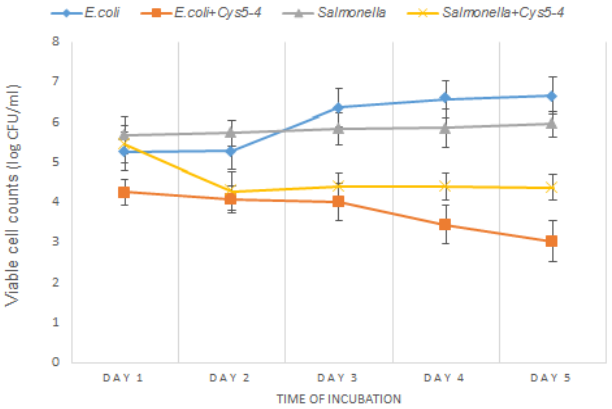

Figure 2. The viability of *E. coli* ATCC 25922 and *Salmonella enterica* ATCC 517411 in fermented orange juice with and without bacteriocin containing CFS Cys5-4 at the room temperature. The error bars indicate standard error of the means of three measurements.

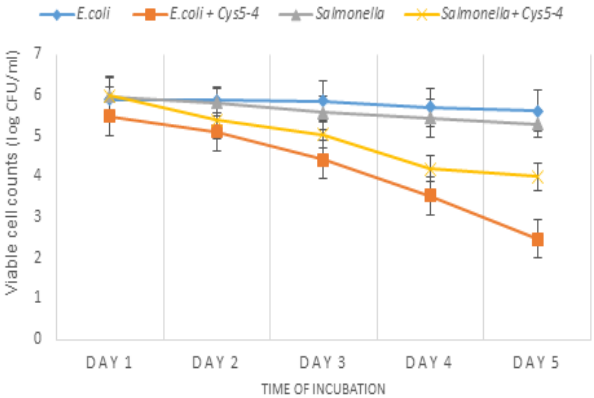

Figure 3. The viability of *E. coli* ATCC 25922 and *Salmonella enterica* ATCC 51741 in fermented chicha juice with and without bacteriocin containing CFS Cys5-4 at 4ºC. The error bars indicate standard error of the means of three measurements.

correlate with our previous observations that acidic condition enhanced the inhibitory activity as well as Cys5-4 exerts its killing action in a bactericidal manner (Garzón *et al*., 2017). This might be associated with a synergistic effect of acids and secreted peptides likewise acids might facilitate the translocation of peptides through the target cell wall (Hartman *et al.*, 2011; Arena *et al.*, 2016). No pathogen was detected in the samples without adding CFS Cys5-4 neither indicator strain. A gradual reduction of *Salmonella enterica* up to Day 2 was detected and the amount of cells was maintained during storage period (Figure 2). In the control (only pathogen added) the viable cell counts did not changed significantly and was maintained between 5.45 to 5.95 log CFU/ml over storage period. These results suggested the efficacy of using CFS Cys5-4 as antimicrobial agent in orange juice. No changes in the juice pH (4.0) was observed after adding bacteriocin indicating that the viability reduction of the target cell is the result of CFS nor acidity. When applied CFS Cys5-4 on chicha juice the cell density of *E. coli* decreased progressively from 5.46 log CFU/ml on Day 1 up to 2.46 log CFU/ml on Day 5 in the samples containing the bacteriocin Cys5-4 (Figure 3). No such reduction level was observed with *Salmonella enterica.* If on Day 1 the pathogen density was 5.9 log CFU/ml, on Day 5 decreased at 4.0 log CFU/ml. In the juice samples where pathogen only was applied the number of viable cells was kept in the range of 6.0 to 5.3l og CFU/ml. Same as in orange juice no changes in the pH occurred, while the indicator cells were smaller than those seen in the positive control. These results suggest that Cys5-4 might bind the cell-wall leading to their destabilization, thus exerting their bactericidal mode of action. Early study demonstrated the effectiveness of Nisin on inhibiting *Listeria* and *E. coli* in fruits and vegetable juices (Abriouel *et al.*, 2003). In other study, a reduction of viable *Listeria monocytogenes* MTCC657 has been shown when applied to pasteurized and unpasteurized fresh orange juice up to Day 4, when the pathogen viability increased significantly (Backialakshmi *et al.*, 2015). Another study on milk and ground beef showed that CFS containing two bacteriocins, sakacin A and sakakin X of *Lactobacillus sakei,* displayed very selective inhibition rates of *Listeria monocytogenes,* where sakacin A was effectively in meat (512AU/g) and sakacin X in whole milk (2048AU/ml) (Hartmann *et al*., 2011). These results implied the importance of testing the efficacy of bacteriocin to which type of food matrix considered to be applied against selected or not-selected targets. Those results showed different inhibition rate; Cys5-4 was more effectively on inhibiting *E. coli* than *Salmonella* in orange juice stored at room temperature as well as chicha stored in refrigeration, suggesting that the target pathogen and drink composition might represent the key factors to assure the preservative effect. For example, when Cys5-4 was applied to chicha at room temperature, the fermentation process extends subsequently with the accumulation of metabolic products (i.e. acid acetic or peroxide) altering the juice quality, thus giving an appropriate environment for *Salmonella* growth. These findings are consistent with other investigations (Ghalfi *et al.*, 2006; Vezcovo *et al*., 2006; Assous *et al*., 2012). More recent study demonstrated the efficacy of bacteriocin ALB101 and ALB103 of *Leuconostoc mesenteroides* on inhibiting *Listeria monocytogenes* in pasteurized and non-pasteurized orange juice (Backialakshmi *et al*., 2015).

Conclusion

A bacteriocin secreted by a native strain *L. plantarum* Cys5-4, showed strong antagonism towards several pathogenic strains. We reported the efficacy of cell-free supernatant containing Cys5-4 bacteriocin to reduce *E. coli* and *Salmonella enterica,* the main pathogenic bacteria founded in artisanal fresh orange juice and chicha beverage demonstrating the promising approach to be integrated as part of overall good manufacturing practice program in those products. It would be recommended to evaluate the efficacy in other food matrix such as raw meat or vegetables.

Acknowledgements

The research was financed by The Technical University of the North, Grant No. 01791. GNT was sponsored in part by the Prometeo Project of Secretary for Higher Education, Science, Technology and Innovation. Authors thanks Clara Ortega, Jimena Suárez, Mishell Rojas and Erika Perugachi for their technical support.

References

Amin, R. A. 2012. Effect of bio preservation as a modern technology on quality aspects and microbial safety of minced beef. Global Journal of Biotechnology and Biochemistry 7 (2): 38-49. doi: 10.5829/idosi.gjbb.2012.7.2.64154.

Abriouel, H., Valdivia, E., Martinez-Bueno, M., Maqueda, M. and Galvez, A. N. 2003. A simple method for semi-preparative-scale production and recovery of enterocin AS-48 derived from E*nterococcus faecalis* subsp. *Liquefaciens* A-48-32. Journal of Microbiological Methods 55 (3): 599-605. https://doi.org/10.1016/S0167-7012(03)00202-1

Assous, M. T. M., Khalaf-Allah, A. M., Sobhy, H. M. and Amani, M. I. H. 2012. Inhibition of *Bacillus cereus* in fresh guava-nectar by plantacirin and nisin. World Journal of Dairy and Food Science 7: 93-100. doi: 10.5829/idosi.wjdfs.2012.7.1.6436

Backialakshmi, S., Meenakshi, R. N., Saranya, A., Jebil, M. S., Krishna, A. R., Krishna, J. S. and Suganthi, R. 2015. Biopreservation of fresh orange juice using antilisterial bacteriocins101 and antilisterial bacteriocin103 purified from *Leuconostoc mesenteroides* Journal of Food Processing and Technology 6: 479. doi:10.4172/2157-7110.1000479.

Banerjee, S. P., Dora, K. C. and Chowdhury, S. 2013. Detection, partial purification and characterization of bacteriocin produced by *Lactobacillus brevis* FPTLB3 isolated from freshwater fish. Journal of Food Sciences and Technology 50(1): 17–25. *http://dx.doi: 10.1007/s13197-011-0240-4.*

Benavidez, A. B., Ulcuango, M., Yepez, L. and Tenea, G. N. 2016. Assessment of the in vitro bioactive properties of lactic acid bacteria isolated from native ecological niches of Ecuador. Revista Argentina de Microbiologia 48(3): 236-244. *http://dx.doi: 10.1016/j.ram.2016.05.003*

Biscola, V., Todorov, S. D., Capuano, V. S. C., Abriouel, H., Gálvez, A. and Franco, B. D. G. M. 2013. Isolation and characterization of a nisin-like bacteriocin produced by a *Lactococcus lactis* strain isolated from charqui, a Brazilian fermented, salted and dried meat product. Meat Science 93: 607-613. http://dx.doi.org/10.1016/j.meatsci.2012.11.021.

Cleveland, J., Montville, T. J. and Nes, I. F. and Chikindas, M. L. 2001. Bacteriocins: Safe, natural antimicrobial for food preservation. International Journal of Food Microbiology 71: 1-20.

de Arauz, L. J., Jozala, A. F., Mazzola, P. G., and Penna, T. C. V. 2009. Nisin biotechnological production and application: a review. Trends in Food Science and Technology 20: 146–154. doi: 10.1016/j.tifs.2009.01.056

Deegan, L. H., Cotter, P. D., Hill, C. and Ross, P. 2006. Bacteriocins: Biological tools for biopreservation and shelf-life extension. International Dairy Journal 16: 1058-1071. *http://dx.doi:10.1016/j.idairyj.2005.10.026*

Delves-Broughton, J. 2005. Nisin as a food preservative. Food Australia 57: 525-527.

Garzón, K., Ortega, C., Tenea, G.N. 2017. Characterization of bacteriocin-producing lactic acid bacteria isolated from native fruits of Ecuadorian Amazon. Polish Journal of Microbiology 66(4):473-481. doi: 10.5604/01.3001.0010.7037.

Ghalfi, H., Allaoui, A., Destain, J., Benkerroum, N. and Thonart, P. 2006. Bacteriocin activity by Lactobacillus curvatus CWBI-B28 to inactivate Listeria monocytogenes in cold-smoked salmon during 4ºC storage. Journal of Food Protection 69: 1066-1071. doi: 10.4315/0362-028X-69.5.1066

Hartmann, H. A., Wilke, T. and Erdmann, R. 2011. Efficacy of bacteriocin-containing cell-free culture supernatants from lactic acid bacteria to control *Listeria monocytogenes* in food. International Journal of Food Microbiology 146(2): 192-199. doi: 10.1016/j.ijfoodmicro.2011.02.031.

Ishiyama, Y., Takata, T., Nakanishi, T., Kaneoke, M., Watanabe, K. -I., Yanagida, F., Chen, Y. -S., Kouya, T., Tanaka, T. and Taniguchi, M. 2008. Production of bacteriocins by several lactic acid bacteria and their application to growth inhibition of spoilage bacteria related to Hiochi. Japan Journal of Food Engineering 9 (4): 277-286.

Keren, T., Yarmus, M., Halevy, G. and Shapira, R. 2004. Immunodetection of the bacteriocin Lacticin RM: Analysis of the influence of temperature and Tween 80 on its expression and activity. Applied Environmental Microbiology 70: 2098-2104. doi: 10.1128/AEM.70.4.2098-2104.2004

Messaoudi, S., Manai, M., Kergourlaya, G., Prévosta, H., Connile, N., Chobert, J. M. and Dousseta, X. 2013. *Lactobacillus salivarius*: Bacteriocin and probiotic activity. Food Microbiology 36 (2): 296–304. doi: 10.1016/j.fm.2013.05.010.

Nath, S., Chowdhury, S., Dora K. C. and Sarkar, S. 2014. Role of biopreservation in improving food safety and storage. Journal of Engineering Research and Applications 4 (1): 26-32.

NTE INEN 2337. 2008. Norma Tecnica Ecuatoriana. Instituto Ecuatoriano de Normalización. Jugos, pulpas, concentrados, nectares, bebidas de Frutas y vegetales. Requisitos, 03 de Agosto de 2008. Quito, Ecuador: Instituto Ecuatoriano Denormalización.

Pratush, A., Gupta, A., Kumar, A. and Vyas, G. 2012. Application of purified bacteriocin produced by *Lactococcus lactis* AP2 as food biopreservative in acidic foods. Annals Food Science and Technology 13: 82-87.

Parada, J. L., Caron, C. R., Medeiros, A. B. P. and Soccol, C. R. 2007. Bacteriocins from lactic acid bacteria: Purification, properties and use as biopreservatives. Brazilian Archives of Biology and Technology 50: 521-542.

Rico, D., Martin-Diana, A. B., Barat, J. M. and Barry-Ryan, C. 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends in Food Science and Technology 18: 373-386. doi:10.1016/j.tifs.2007.03.011

Sperber, W. H. 2009. Introduction to the microbiological spoilage foods and beverages. In Sperber, W.H and Doyle, M. P. (Eds). Compendium of the microbiological spoilage 1 of foods and beverages. Food Microbiology and Food Safety, p. 1-40. New York: Springer. doi: 10.1007/978-1-4419-0826-1.

Taheri, P., Samadi, N., Ehsani, M. R., Khoshayand, M. R. and Jamalifar, H. 2012. An evaluation and partial characterization of a bacteriocin produced by *Lactococcus lactis* subsp *lactis* ST1 isolated from goat milk. Brazilian Journal of Microbiology 43(4): 1452–1462. doi: 10.1590/S1517-838220120004000029.

Tenea, G. N. and Yepez, L. 2016. Bioactive compounds of lactic acid bacteria. Case study: Evaluation of antimicrobial activity of bacteriocin-producing lactobacilli isolated from native ecological niches of Ecuador. In Venketeshwer, R. (Ed.) Prebiotics and probiotics in human nutrition and health, p. 147-169. InTech (Open Access). Doi: 10.5772/63112.

Teusink, B., Wiersma, A., Jacobs, L., Notebaart, R. A. and Smid, E. J. 2009. Understanding the adaptive growth strategy of *Lactobacillus plantarum* by in silico optimisation. PLoS Computational Biology 5(6): e1000410. doi:10.1371/journal.pcbi.1000410.

Todorov, S. D., Gotcheva, B., Dousset, X., Onno, B. and Ivanova, I. 2000. Influence of growth medium on bacteriocin production in *Lactobacillus plantarum* ST31. Biotechnological and Biotechnological Equipment 14: 50-55.

Todorov, S. D. 2009. Bacteriocins from *Lactobacillus plantarum*-production, genetic organization and mode of action. Brazilian Journal of Microbiology 40: 209-221. doi: 10.1590/S1517-83822009000200001.

Todorov, S. D., Vaz-Velho, M., de Melo, G., Franco, B. D. and Holzapfel, W. H. 2013. Partial characterization of a bacteriocin produced by three strains of Lactobacillus sakei isolated from salpicao, a fermented meat product from North-West of Portugal. Food Control 30: 111-121. *http://dx.doi.org/*10.1016/j.foodcont.2012.07.022.

Vescovo, M., Scolari, G. and Zacconi C. 2006. Inhibition of *Listeria innocua* growth by antimicrobial-producing lactic acid cultures in vacuum-packed cold-smoked salmon. Food Microbiology 23: 689-693. doi: 10.1016/j.fm.2005.12.002.

Xin, B., Zheng, J., Xu, Z., Li, C., Ruan, L., Peng, D. and Sun. M. 2015. Three novel lantibiotics, Ticins A1, A3, and A4, have extremely stable properties and are promising food biopreservatives. Applied Environmental Microbiology 81 (20): 6964-6972. doi: 10.1128/AEM.01851-15.

Zamfir, M., Callewaert, R., Cornea, P. C., De Vuyst, L. 2000. Production kinetics of acidophilin 801, a bacteriocin produced by *Lactobacillus acidophilus* IBB 801. FEMS Microbiology Letters 190: 305–308. doi: 10.1111/j.1574-6968.2000.tb09303.x

Winkelstroter, L. K., Gomes, B. C., Thomaz, M. R. S., Souza, V. M. and De Martinis C. P. 2011. *Lactobacillus sakei* 1 and its bacteriocin influence adhesion of *Listeria monocytogenes* on stainless steel surface. Food Control 22: 1404-1407. doi: 10.1016/j.foodcont.2011.02.021.