BACTERIOCIN EFFECT OF LACTIC ACID BACTERIA ISOLATED FROM HIGH PRESSURE TREATED TURKEY HAM AGAINST *SALMONELLA ENTERITIDIS*

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Abstract. *Lactic acid bacteria (LAB) are widely used in technological processes for the fermentation of vegetables and meat products. However, some LAB comprise the deteriorative micro flora of vacuum packaged meat products, being responsible for the production of compounds determinant of undesirable characteristics of color, odor and appearance. More recently LAB have been studied due to the production of bacteriocins, raising great interest in the food industry for their potential as biopreservatives. This study aimed at isolating and identifying LAB presented in stored turkey ham (7 °C) processed by high hydrostatic pressure (400 Mpa/15 min.) and evaluating their potential in producing bacteriocins. It also aims at evaluating the efficacy of the bacteriocins against Salmonella Enteritidis. LAC from laboratory formulated and processed turkey ham were isolated in MRS agar media and M-17, through identification of genera by biochemical tests, Gram staining and catalase assay. It was concluded that the genera of both LAB isolated from the pressurized and stored turkey ham were Enterococcus and Streptococcus. Regarding the production and efficacy of bacteriocins, the majority of Enterococcus colonies originated from both pressurized and non-pressurized turkey ham formed inhibition zone related against Salmonella Enteritidis. In the case of Streptococcus, only the colonies originated from the non-pressurized turkey ham resulted in bacteriocinic effect, while the colonies originated from the pressurized turkey ham did not show inhibition zone against Salmonella Enteritidis.*

Keywords. Lactic acid bacteria, bacteriocins, high hydrostatic pressure, turkey ham.

Introduction

Lactic acid bacteria constitute a microbial group broadly distributed in different foods, either as contaminant or intentionally added microorganisms, being able to produce a variety of antimicrobial compounds such as: acids, dyacetil, hydrogen peroxide, carbon dioxide, alcohol, aldehyde and bacteriocins. All those compounds may antagonise deteriorative and pathogenic bacteria present in food (Hugas, 1998; Schillinger & Lücke, 1989), and because of that it has been raising the interest of food industry to the potential use as natural preservative (Cleveland, Montville, Nes & Chikindas, 2001).

LAC microbiota constitutes by a heterogenic group of bacteria from different genus with different physiological demands, which includes among them *Enterococcus, Lactococcus, Pediococcus, Leuconostoc, Weisella* and *Lactobacillu*s. In common they have the characteristics of being positive Gram, negative catalase, non spore forming, facultative anaerobic, well adapted to rich nutrient environment and produce lactic acid as the main fermentation product from glicides (Axelsson, 1993).

According to Caplice & Fitzgerald (1999), bacteriocines producing bacteria are often present and can be isolated from LAB containing foods, such as meat and dairy products. Shillinger & Lücke (1989) consider that LAB originally isolated from meat and meat products are the most appropriate microorganisms to be used aiming at enhancing the food safety of that type of food. According to those authors such bacteria are well adapted to the meat and meat products conditions and may as a consequence be more competitive in comparison to the LAB originated from other sources. Bacteriocines can act synergistically together with traditional food preservation hurdles or with any other new preservation technology that may lead to a sub lethal injury. That is the case of high hydrostatic pressure (HHP) which is an innovative emerging technology that allows the benefit of reducing microbial load while preserving food sensory and nutritional characteristics (Rastorgi Raghavarao, Balasubramaniam, Niranjan & Knorr, 2007). According to Kalshayanand, Sikes, Dunne & Ray (1994); Ter Steeg, Hellemons & Kok (1999), HHP increase the external membrane permeabilisation in Gram negatives which enhances bacteriocin penetration and its antimicrobial efficiency. So far it still limited the research regarding the combined effect of HHP and bacteriocines (Aymerich, Jofré, Garriga & Hugas 2005).

The present study aimed at isolating, identifying the genus and qualitative evaluate the inhibition activity against *Salmonella* Enteritidis in turkey ham and treated with HHP.

Material and methods

Turkey ham preparation and HHP tretament

Turkey ham processing comprehended first the turkey tight toilet in order to remove tendons, nerves, skin and bones.

Additives and condiments immersed in brine were added together with the turkey meat pieces into the cutter (*Geiger* model UM12) and processed up to a homogeneous mass

was obtained, followed by cold storage at 7 °C for 24 hours. In the sequence the mass was packed in temperature resistant plastic packages (cook-in), thermally vacuum sealed and inserted into steel container for the cooking up to the product centre reached 72 °C. The product was then chilled at 4 °C for 24 hours and samples were collected and destined to laboratory analyses.

High hydrostatic treatment applied to pre-packed sliced turkey ham was carried out in a laboratory scale unit (*Stansted Fluid Power*, S-FL-850-9-W model) at 400 MPa for 15 min. at room temperature based on Slongo *et al.* (2009).

Lactic Acid Bacteria isolation and identification

Lactic Acid Bacteria isolation and growth evaluation was performed according Hall *et al.* (2001), by pour-plating serial diluted solutions on de Man, Rogosa, Sharp-MRS Agar (Oxoid) and M-17 Agar (Fluka Analytical), followed by BOD incubation at 30 °C for 3 to 5 days. Analyses were carried out in duplicate. Following the growth in the isolation media, colonies with well-defined characteristics were selected and replicated for the media used for the genus identification, according to methodoly used by Harrigan (1998) together with catalase test, the methodology established by Vanderzant & Splittstoesser (1992), besides Gram colouration.

Antimicrobial activity determination

Direct inhibition test was carried out according to Santos (1993), in which a culture of Enteritidis ATCC 13706 chilled and maintained in TSA Agar (Difco) was transferred to brain and heart infusion (BHI) broth and incubated at 35 °C for 12 hours. Selected colonies were plated in agar MRS and M-17 and further incubated 35 °C for 2 hours. From the tubes containing the initiating cultures of indicator microorganism an aliquot was transferred to another tube containing BHI broth and the procedure repeated again taking the sample from the inoculated BHI broth tube this time. 750 µL of the former tube were transfer to a tube containing 10 mL of 0.87% BHI Ágar (Oxoid) which was prepared with BHI broth added with Agar-Agar (Merck). 23067999 75137407 The pre-prepared content was maintained liquefied in water bath at 37 °C and then poured into the plates containing the LAC followed by incubation at 35 °C for 24 hours. At the end of the procedure the presence or absence of inhibition haloes was verified.

Results and discussion

Among 108 isotalte colonies 77.7% were positive Gram and 66,6% negative catalase. Taking into account the LAC identification tests only 72 colonies remained to be confirmed which were destined to the genus identification test, according to the methodology described by Harrigan (1998). The results of identification tests are summarised in Table 1.

Table 1. Shape of bacteria isolated from turkey ham in MRS and M-17 media, Gram coloration and negative catalase test negativa for LAB genus identification

Isolated bacterias shape	Growth in MRS	Growth in M-17	Gram +	Catalase -
Curved (gout)	+	+	+	+
Flat	+	-	-	-
Round	+	-	+	-
Small round	+	-	-	-
Puntiforme	-	+	+	+

Among those, 48 (66.6 %) colonies were classified as belonging to the *Enterococcus spp.,* genus. Such a classification had as the criteria the fact that the microorganism were non spore forming positive Gram cocos, presenting negative catalase, being homofermentative, with growth capacity at 10 and 45 °C, 5% NaCl and pH 4.4 and 9.6. The 24 remaining colonies were identified as *Streptococcus spp.*, being negative catalase positive Gram cocos, homofermenative and with growth capacity at 45 °C but not at 10 °C, in the presence of 6.5% NaCl and in a pH 4.4 and 9.6 (Harrigan, 1998; Axelsson, 1993; Holt *et al.*, 1994).

Figure 1 present micrographs of *Enterococcus sp* and *Streptococcus sp* obtained from LAB isolated microrganisms, showing (a) oval cocos and grouped cocos (b), respectively.

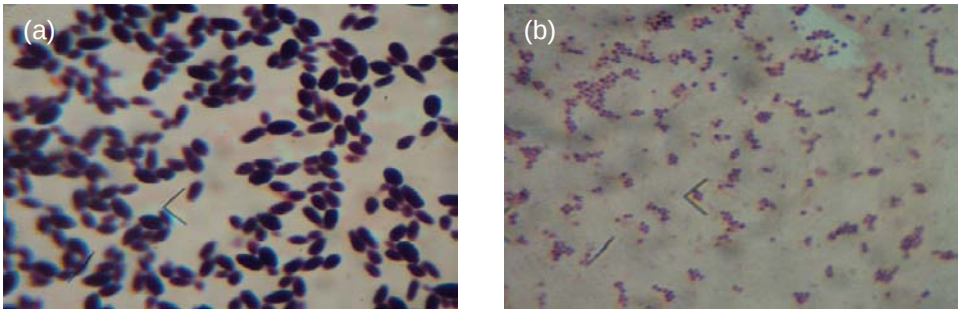

Figure 1. Morfology of (a) *Enterococcus sp* and (b), *Streptococcus sp* identified with immesrion objective.

The genus *Enterococcus spp.* was isolated and submitted to the inhibition tests against *Salmonella* Enteritidis. Among 48 colonies 24 were obtained from the control sample and shown 91.6% inhibition haloes formation. Regarding the 24 colonies isolated from

pressurised turkey ham, the inhibition haloes was verified in 70.8% of them. Besides, the inhibition haloes were smaller in the case of the pressurised turkey colonies in comparison to the control ones.

In the case of the colonies originated from the *Streptococcus spp.*, 12 colonies were obtained from control turkey ham from which only 25.0% formed the inhibition halos. Among the 12 colonies originated from the pressurised turkey ham just 8.3% resulted in the inhibition halos. Both cases resulted in small inhibition halos. The results showed a limited growth of the *Streptococcus sp*, and a limited production of the tested bacteriocin activity.

The inhibition halos proved to be lower the inhibition activity of the LAC originated from pressurised samples in comparison to the non-pressurised (control) ones, possibly due to the modification in the cellular metabolism which possibly lead to the decrease in the antimicrobial compounds.

Conclusions

LAB genuses isolated from turkey ham were *Enterococcus sp* e *Streptococcus sp*. The possible contamination origin was from the processing operations including cutting, slicing and raw material and product manipulation. In spite of belonging to a deteriorating microbiota, it has been important to isolate and identify which LAB compose the turkey ham microbiota and evaluate if they may induce the competition and pathogen inhibition, such as the evaluated *Salmonella*, since they are often regarded as an inhibition hurdle that contribute to the shelf life extension in addition to the high pressure.

The inhibition halo was detected in most *Enterococcus spp.* isolated mainly originated from control ham, and less frequent and with lower intensity in the *Streptococcus sp.* isolates. Future studies are necessary for a better understanding of such specific bacteriocins compounds on pathogenic microorganisms and in which way these are affected by high pressure.

References

Aymerich, M.T.; Jofré, A.; Garriga, M.; Hugas, M. 2005. Inhibition of *Listeria monocytogenes* and *Salmonella* by natural antimicrobials and high hydrostatic pressure in sliced cooked ham. *Journal of Food Protection* 68: 173–177.

Axelsson, L.T. 1993. *Lactic acid bacteria: classification and physiology*. In: *Lactic acid bacteria*, pp.63, Salminen, S., & Wright, A, ed. New York: Marcel Dekker.

Caplice, E., & Fitzgerald, G.F. 1999. Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology* 50: 131-149.

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

Hugas, M.1998. Bacteriogenic lactic acid bacteria for the biopreservation of meat and meat products. *Meat Science* 49: S139-S150.

Kalchayanand, N., Sikes, T., Dunne, C.P., & Ray, B. 1994. Hydrostatic pressure and electroporation have increased bactericidal efficiency in combination with bacteriocins. *Applied and Environmental Microbiology* 60: 4174–4177.

Rastogi, N.K., Raghavarao, K.S.M.S., Balasubramaniam, V.M., Niranjan, K. & Knorr, D. 2007. Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition* 47: 69–112.

Shillinger, U., & Lücke, F.K. 1989. Antibacterial activity of Lactobacillus sake isolated from meat. *Applied and Environmental Microbiology* 55: 1.901-1.906.

Slongo, A.P., Rosenthal, A., Camargo, L.M.Q., Deliza, R., Mathias, S.P., & Aragão, G.M.F., 2009. Modeling the growth of lactic acid bacteria in sliced ham processed by high hydrostatic pressure. Food Science and Technology, 42, 303-306.

Ter Steeg, P.F.; Hellemons, J.C.; Kok, A.E. 1999. Synergistic actions of nisin, sublethal ultrahigh pressure, and reduced temperature on bacteria and yeast. *Applied and Environmental Microbiology* 65: 4148–4154.