EFFECT OF BACTERIOCINS PRODUCED BY *LC. LACTIS* SUBSP. *CREMORIS* CTC 204, *ENTEROCOCCUS AVIUM* CTC 483, AND *LC. LACTIS* SUBSP. *HORDNIAE* CTC 484 ON MESOPHILLIC AEROBIC COUNTS OF MINCED BEEF DURING REFRIGERATED STORAGE

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Background

The surface of fresh meat is contaminated with a number of microorganisms, even when it has been produced under good hygiene conditions (SCHILLINGER & LÜCKE, 1987). The initial microflora of meat contains both mesophillic and psychrotrophic bacteria (GILL & NEWTON, 1997), which are the most important factors in determining the composition of spoilage flora and its growth rates at chill temperature. Indeed, the predominance of lactic acid bacteria in the microflora of fresh and processed meat is well known (BORCH *et al.*, 1996; LAMBERT *et al.*, 1991). These bacteria are able to preserve meats by competitive exclusion of other microorganisms, because of their ability to grow at refrigerator temperatures, their resistance to the effects of carbon dioxide on the bacterial cell, and also by the production of antimicrobial substances, including hydrogen peroxide, acetaldehyde, organic acids, and bacteriocins (McMULLEN & STILES, 1996). Bacteriocins are naturally produced peptides that are antagonistic to other closely related bacteria. These compounds play an important role in the ability of lactic acid bacteria to prevail in mixed populations (KLAENHAMMER, 1993). The potential use of bacteriocin-producing lactic acid bacteria and their bacteriocins as biopreservative in meat is immense and has not been exploited by the meat industry.

Objectives

The purpose of this study was to investigate the effect of three bacteriocins, produced by strains of lactic acid bacteria isolated from meats, on the mesophillic population presented on minced raw beef.

Methods

Bacterial strains and media

The bacteriocin-producing strains of lactic acid bacteria and the indicators microorganisms were from the Centro de Tecnologia de Carnes, Instituto de Tecnologia de Alimentos, Brazil (CTC-ITAL) culture collection: *Lactococcus lactis* subsp. *cremoris* CTC 204 isolated from gizzard; *Enterococcus avium* CTC 483 and *Lactococcus lactis* subsp. *hordniae* CTC 484 both isolated from chicken liver. *Listeria innocua* Lin 11 (Institute Pasteur, Paris, France) and *Staphylococcus aureus* CTC 033 (ITAL, Campinas, S.P., Brazil) were used as indicator cultures. Stock cultures of lactic acid cultures were stored at –80°C in Man, Rogosa, Sharpe Broth (MRS, Oxoid CM359) supplemented with 15% (v/v) glycerol, whereas the indicators were kept at refrigeration temperature (4°C) on Tryptone Soya Agar (TSA, Oxoid CM131). Before use, cultures were transferred twice in MRS or Tryptone Soya Broth (TSB, Oxoid CM129) and incubated at 30°C for 24 h.

Bacteriocin preparation

Cell-free supernatants from bacteriocin-producing strains were collected by centrifugation (12,000 rpm/10 min, 4°C) of MRS Broth cultures. The supernatant fluids were neutralized to pH 6.5 with 10 N NaOH and sterilized by heating at 100°C for 5 min. These crude bacteriocin preparations were kept at 5°C until use.

Bacteriocin activity assay

The activities of crude bacteriocin preparations were determined by the critical dilution assay of MAYR-HARTING *et al.* (1972). The title was defined as the reciprocal of the highest dilution showing an inhibition of the indicator strain multiplied by 100 to express the results as activity units by milliliter (AU/mL). Bacterial numbers were calculated as log cfu/g.

Meat preparation and inoculation

Twenty-five-g portions of minced beef were dipped into bacteriocin preparation during 5 min following draining for 2 min. The samples were packed in plastic bags and stored at 4°C. Samples not added of bacteriocin were used as controls. Triplicates were sampled at selected times. For the microbiological determinations, each sample was homogenized with 225 ml of 0.1% peptone water, using a Stomacher (Seward Laboratory, Model '400', London, England). Further decimal dilutions were prepared using 0.1% peptone as diluent. The dilutions were plated, in duplicate, on MRS Agar (Oxoid, CM361) for lactic acid bacteria enumeration on Agar Plate Count (APC, Merck 1.05463) for total mesophillic counts. Inoculated plates were incubated aerobically at 35°C for 48 h. At each sampling, the pH of the samples was monitored using a pH meter (Toledo Mettler, MP125, Switzerland).

Results and Discussion

This study demonstrated that the reduction of the mesophillic population on minced raw beef treated with crude bacteriocin preparations was dependent on the strains, initial contamination of the meat, and storage period. *Lactococcus lactis* subsp. *hordniae* CTC 484 produced the most active bacteriocin (Figure 1A), inducing decrease in aerobic mesophillic microflora up to 2 log cycles compared with the control without bacteriocin. However, this difference was not maintained during the whole storage period. On the other hand, the bacteriocins produced by *Lactococcus lactis* subsp. *cremoris* CTC 204 (Figure 2) and *Enterococcus avium* CTC 483 (Figure 3) had a lower inhibitory effect than the one produced by *Lactococcus lactis* subsp. *hordniae* CTC 484. These bacteriocins caused reductions up to 0.5 cycle in aerobic mesophillic counts. The bacteriocins had an activity of approximately 500 AU/mL The bacteriocin activity was not influenced by the pH values. The greater inhibitory effect of the bacteriocin produced by strain *Lactococcus lactis* subsp. *hordniae* CTC 484 was observed where the initial contamination of the meat was lower. There is little information on the effect of lactic culture treatment and their bacteriocins on mesophillic aerobic plate counts of meat during refrigerated storage. Cell suspensions of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* have been used to inhibit the growth of gram-negative bacterial populations in refrigerated ground beef (DALY *et al.*, 1972). Meat storage in air is rapidly

spoiled by bacteria which are responsible for discoloration and off odours, causing its rejection (LABADIE, 1999). The color of treated and control samples was verified during the period of storage. The formation of green color was detected in the control samples in the end of the storage time. The green fluorescent pigment (pyoverdin) is produced by many fluorescent species of *Pseudomonas* (LABADIE, 1999). Generally, storage life depends largely on the quantities of bacteria on meat at the beginning of the storage and noticeably the proportion of *Pseudomonas* spp. within the flora (DAINTY & MACKEY, 1992).

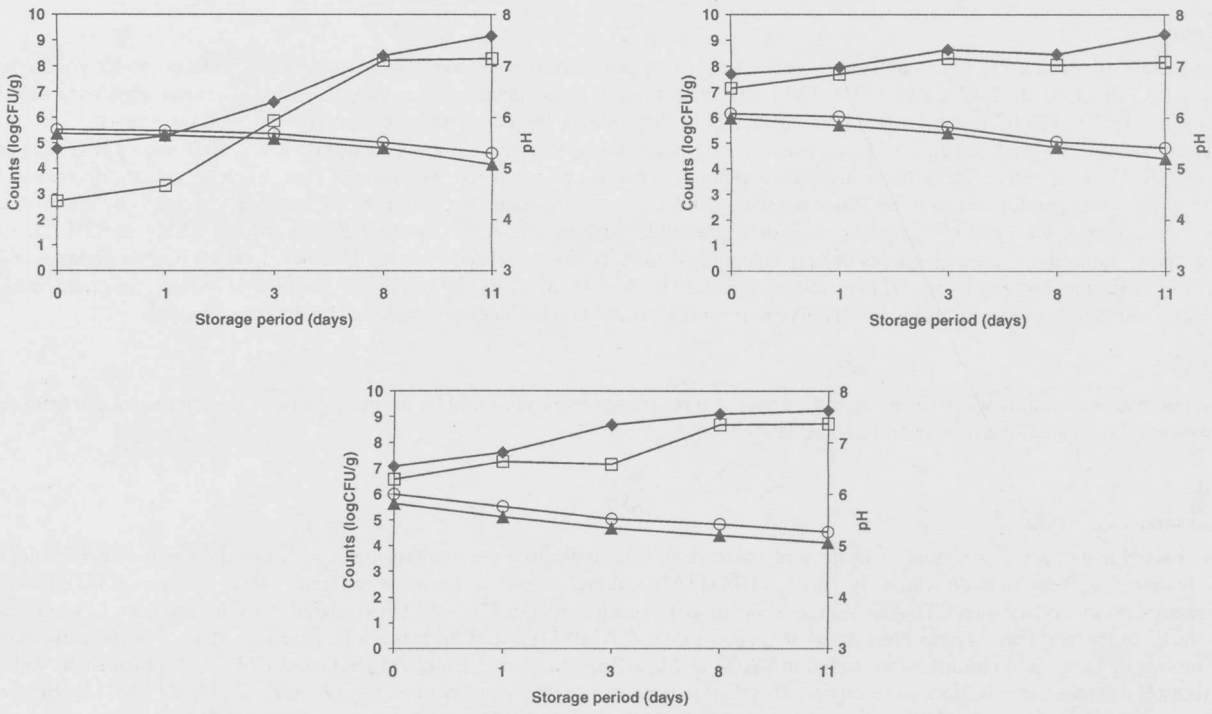

Figure 1. Effect of *Lactococcus lactis* subsp. *hordniae* CTC 484 (A), *Lactococcus lactis* subsp. *cremoris* CTC 204 (B) and *Enterococcus avium* CTC 483 on mesophillic counts of minced beef during refrigerated storage. (□ Treatment counts, ◆ Control counts, ▲ Treatment pH, ○ Control pH).

Conclusion

On the basis of our results it could be concluded that *Lactococcus lactis* subsp. *hordniae* CTC 484 produced the most effective bacteriocin. To achieve a more effective elimination of contaminant strains, a synergistic use of two bacteriocins should also be considered.

Pertinent literature

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