# BACTERIOCIN-LIKE INHIBITOR OF *LACTOCOCCUS LACTIS* SUBSP. *HORDNIAE* CTC 484 AGAINST *LISTERIA MONOCYTOGENES*

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## Background

Bacterial foodborn pathogens, including *Listeria monocytogenes*, have had a dramatic impact on public opinion of the most critical food-related risk factors affecting consumers. Bacteriocins are a member of antimicrobial substances produced by lactic acid bacteria (KLAENHAMMER, 1988). As these compounds have potential applications as natural food preservatives (KRÖCKEL, 1999) diverse bacteriocins have been recently identified and characterized (CLEVELAND *et al.*, 2001). Bacteriocins are considered a "hurdle" to food preservation and safety. In this sense, these compounds can serve as bactericidal barriers and help to reduce the levels of susceptible microorganisms. Literature has demonstrated inhibition of *L. monocytogenes* strains by bacteriocin-producing lactic acid bacteria (MURIANA, 1996). *Lactococcus lactis* subsp. *hordniae* CTC 484, isolated from chicken liver, produces a wide spectrum bacteriocin with activity against Gram-positive pathogenic and meat spoilage microorganisms. The ability of this bacteriogenic strain to act as bioprotection system was examined.

## Objectives

The purpose of this study was to determine whether the bacteriocin-like substances produced by *Lc. lactis* subsp. *hordniae* CTC 484 are active against *L. monocytogenes* in sterile ground beef.

## Methods

Origin and maintenance of the cultures

*Lc lactis* subsp. *hordniae* CTC 484 isolated from natural chicken liver was used as bacteriocin-producing strain. *L. monocytogenes* CTC 021, from the Centro de Tecnologia de Carnes, ITAL, Campinas, Brazil, was used as indicator culture. The lactic acid culture was kept at –80°C in de Man, Rogosa, Sharpe Broth (MRS, Oxoid CM359) supplemented with 15% (v/v) glycerol, whereas *L. monocytogenes* was kept at refrigeration temperature (4°C) on Tryptone Soya Agar (TSA, Oxoid CM131).

Microbiological challenge tests

Fresh lean beef muscle was obtained from a local abattoir, minced, autoclaved and stored at 121°C for 15 min. Plastic bags containing a 25g-portion of sterile ground beef were prepared and inoculated with either *L. lactis* subsp. *hordniae* at 2% (v/v) and *L. monocytogenes* at 1% (v/v). The inoculated samples were stored at 4° and 25°C for up to 16 days. Samples inoculated only with the producer or the indicator were used as positive or negative controls, respectively. Triplicates of each treatment were sampled at selected times. For the microbiological determination, each sample was homogenized with 225 ml of 0.1% peptone water, using a stomacher. Further decimal dilutions were prepared using 0.1 % peptone as diluent. Survival was monitored by enumeration of lactic acid bacteria on MRS Agar (Oxoid, CM361) and of *L. monocytogenes* on Listeria Selective Agar (Oxoid, CM856), supplemented with Listeria Selective Supplement (Oxoid, SR140), following incubation at 30° and 37°C for 48 h, respectively. Bacterial numbers were calculated as log cfu/g. At each sampling, the pH of the samples was monitored using a pHmeter (Toledo Mettler, MP125, Switzerland).

## Results and discussion

The initial number of *L. monocytogenes* in the inoculated ground beef was 6.7 $\log_{10}$ cfu/g at 4°C (**Figure 1**). The effect of the presence of the bacteriogenic strain of lactic acid bacteria was not significant on the 3° day, when the counts of *L. monocytogenes* and the control (without lactic acid bacteria) were both the same: 7.1 $\log_{10}$ cfu/g. Although on the 6° day the growth of *Listeria* in the presence of *L. lactis* subsp. *hordniae* CTC 484 reached 7.7 $\log_{10}$ cfu/g, a difference 0.6 log was found compared with the control. During the second and third weeks high levels of *L. monocytogenes* were observed but the effect of bacteriocin was still significant.

At 25°C (**Figure 2**), the initial counts of *L. monocytogenes* reached 7.0 $\log_{10}$ cfu/g. On the 3° day of storage, a difference 1.0 log was found compared with the control. The effect of the bacteriocin produced by strain CTC 484 was not maintained on the 6° day, when the counts were approximately the same for both samples: test and control (9.0 $\log_{10}$ cfu/g). Nevertheless, from that point on, the inhibitory effect was noticed again: a difference 0.8 and 1.0 log was found compared with the control on the 9th and 13tho days, respectively.

The pH value at the end of experiments was 5.8 in all samples. The pH effect was not significative in the tests.

A variety of factors can prevent the growth of microorganisms, one of which is competition by lactic acid bacteria present in these products (McMULLEN & STILES, 1996). The ability of nisin, the well-known bacteriocin, to control de growth of pathogens and spoilage organisms in fresh and cooked meats is limited (CHUNG *et al.*, 1989; EL-KHATEIB, *et al.*, 1993). The poor performance of nisin as a biopreservative in meats systems has resulted in the search for other bacteriocin-producing lactic acid bacteria. In some cases the bacteriogenic microorganisms used do not grow in meats stored at chill temperatures. According to DEGNAN *et al.* (1992), *Pediococcus acidilactici* JBL1095 produces pediocin AcH/PA-1 in vacuum-packed wieners when the packages are stored at 25°C, but when they were stored at 4°C, the bacterium did not grow and therefore the bacteriocin was not produced. In this research temperature abuse (25°C) has been applied to the meat product studied in order to simulate abusive storage conditions. In adequate refrigeration temperatures (4°C), inhibition of *Listeria* was higher. Also, we should consider that in lower initial inoculum the antilisterial activity would probably have been higher. According to MURIANA (1996), the relatively large size of the bacteriocins and their proteinaceous character may make them susceptible to biochemical reactions involving amino acid side chains or hydrophobic interactions that may interfere with their intended interaction with target cells. Most applications of bacteriocins have produced reductions of 1 to 3 log cycles in *L. monocytogenes* populations in foods. Although these levels are not acceptable as a primary preservation method, they are useful in the "hurdle" concept for reduction of foodborn pathogens.

## Conclusions

*L. lactis* subsp. *hordniae* CTC 484 appears to be a bioprotective culture against *L. monocytogenes* in heat-treated meat products.

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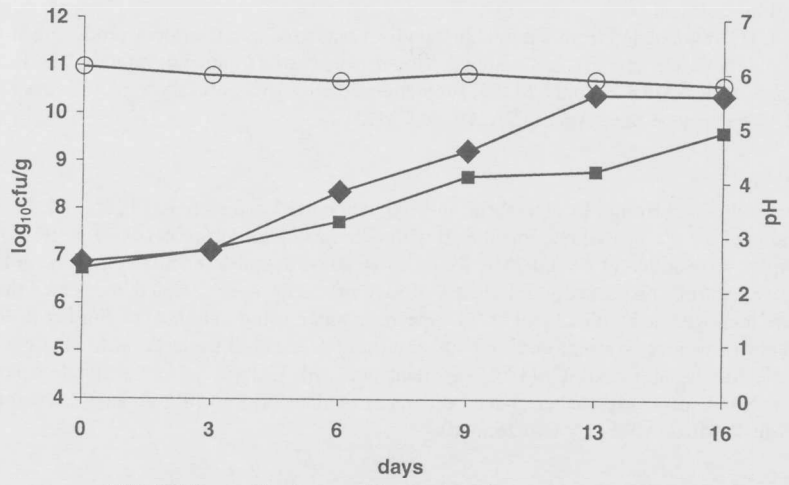


**Figure 1.** Growth of *L. monocytogenes* CTC 021 in sterilized ground beef with (◆) or without (■) *Lc. lactis* subsp. *hordniae* CTC 484 at 4°C and pH (○).

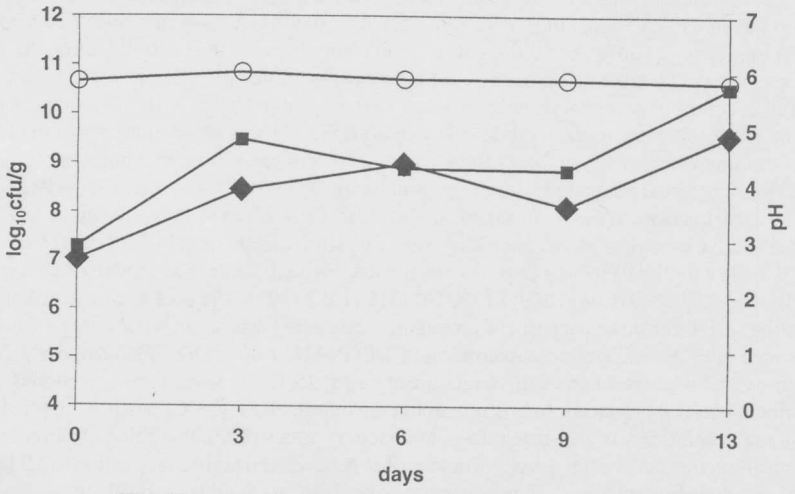


**Figure 2.** Growth of *L. monocytogenes* CTC 021 in sterilized ground beef with (◆) or without (■) *Lc. lactis* subsp. *hordniae* CTC 484 at 25°C and pH (○).