APPLICATION OF A BACTERIOCIN AS A NATURAL PRESERVATIVE IN SAUCES

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SUMMARY

Bacteriocins obtained from Lactic Acid Bacteria (LAB) isolated from different meat products have been proposed, frequently, as a putative good natural preservative, in meat products. The aim of this work is to prove the effectiviness of this kind of substances in a food system. In many ready-to-eat meals, sauces usually are an important part of the dish covering the main component, which often is based on meat. In these foods sauces, according to their characteristics and composition, could be a good way to protect the main component that they usually cover.

A crude extract of a bacteriocin from a *Lactobacillus* strain isolated from Spanish fermented dry-sausage was used. The sauce was autoclave-sterilized, in order to avoid the initial contamination, and afterwards was inoculated with a sensitive target strain (*Enterococcus faecalis*). This mixture was used as a control. Three different treatments were compared to test their influence on the shelf-life of the sauce: to the control were added bacteriocin crude extract (BCE) or this extract and 1% of sodium lactate or 1% sodium lactate. The evolution of the sensitive strain population kept at 15°C, was followed for the three treatments. The results obtained show that the sauce with the BCE or the one with sodium lactate alone were effective for around 10 days. However, when both products were used together, a synergic effect was showed and the sensitive strain population disappeared from the sauce. According to the results, the sauce could be a good vehicle for bacteriocins developing their bacteriocidal or bacteriostatic power.

Introduction

Bacteriocins from LAB have been proposed as a putative good preservative in food systems by their bacteriocidal or bacteriostatic activity against food spoilage bacteria and foodborne pathogens. They usually attack to closely related species of the producer strain but many of them can avoid the growth of *L. monocytogenes*. They also have the advantage that they are usually bacteriocidal, and are produced by GRAS (General Recognized As Safe) microorganisms, that in most of the cases have been isolated from a natural food system.

Bacteriocins are quite lipophylic and they usually have the cell membrane of the sensitive strain as a target. It has been proposed that bacteriocins have a common mode of action, which lies in dissipating the membrane potential (Hanlin et al. 1992, Bruno et al. 1993, Nettles et al. 1993, Nes et al. 1993). Most of the bacteriocins are heat resistant and all of them are proteinaceous in nature (are degradated by the organism proteases). All these characteristics are of great importance in order to consider bacteriocins as a good candidate for biopreservation in food systems.

However, some difficulties must be taking in account. It seems that in solid foods the diffusion of the bacteriocin is a great problem (Schillinger et al. 1991). They also could be adsorbed by the lipid component of the food. They are not effective against spoilage and pathogenic Gram-negative bacteria, yeast and molds. They usually have a quite narrow spectrum and they are also strain specific, that means there are insensitive variant cells which can grow in the presence of bacteriocin, even in sensitive Gram-positive strains, (Hanlin et al, 1992). These limitations have to be overcome to be an effective food preservative.

In the last few years, some works have appeared, that try to prove the effectiviness of bacteriocines in food systems (Nettles et al. 1993, Nes 1993). In this study, a bacteriocin isolated from a meat product is used to extent the shelf-life of a sauce of a ready-to-eat meal. These kind of food products are increasingly demanded by the consumer. Traditionally, these products have been sterilized. These methods, which are very safe from the microbiological point of view, have some disadvantages for the consumer. They usually change the

sensorial properties of foods and also are contrary to the concept of "freshness" which demands the consumer. For these reasons, pasteurized ready to eat meals could be a good alternative, and a natural preservative method. So that, the use of bacteriocins could be very interesting in order to extend the shelf-life of these products.

Material and Methods

Preparation of Bacteriocin Crude Extract

A bacteriocin produced by a *Lactobacillus sake* strain MG 238 was used. This strain was isolated from Spanish fermented dry-sausage (Rodriguez et al, 1993). In order to obtain the bacteriocin, two overnights cultures from a vial were done. The cultures were grown in MRS broth (Oxoid) at 30°C in anaerobic contidions. After the second overnight, a 24 hours culture were done in the same conditions. In this latter case a 100 ml flask of MRS broth was used to get enough amount of bacteriocin. A centrifugation step at 8.000g for 25 minutes was intercalated. Supernatant was collected and neutralized with NaOH 1N, filter-sterilized and keep in refrigeration till it was used. This neutralized and filter-sterilized supernatant was called Bacteriocin Crude Extract (BCE).

Bacteriocin Crude Extract Activity

The activity of the BCE was checked according to the following method. A MRS agar containing only 0.2% of glucose (MRS 0.2) (Scillinger et al, 1983) was overlaid with a soft brain heart infussion (BHI) agar (containing 0.7% agar) inoculated with approximately $5 \cdot 10^7$ cells of the indicator strain *E. faecalis*. When the overlaid agar was dry, one drop (10 ul) of twofold serial dilutions of the supernatant were spotted onto the surface of the agar plates. The drops were let dry and then were incubated overnight at 30°C in anaerobic conditions. The activity was calculated from the inverse of the last dilution that shows a clear zone of inhibition (2mm in diameter or more) in the agar plate, and it was expresed in Activity Units per ml (AU/ml) (Barefoot et al, 1983).

Treatments with the Sauces

A type of pepper sauce based on pepper, tomato and some gellificans was chosen to prove the effectiviness of the bacteriocin from *L. sake* MG 238. 25 ml of pepper sauce were introduced in a flask and were autoclave-sterilized (20 minutes at 121°C). When the flasks were cool the following trials were done:

1. One flask with only pepper sauce.
2. A second flask with sauce was inoculated with 0.5 ml of an overnight culture of the indicator strain *E. faecalis* (approximately $5 \cdot 10^3$ CFU/ml of sauce)(Control flask).
3. Another flask were prepared as the second one, but 2.5 ml of BCE were added (Bac+ flask).
4. A fourth fask were prepared as the second one, but 0.25 ml of sodium lactate (1%) were added (L+ flask).
5. The last flask were prepared as the third one, but 0.25 ml of sodium lactate (1%) were also added (BacL flask).

All flasks were incubated at 15°C for 31 days and, through out incubation, samples were taken at appropiate intervals. The number of viable cells was determined by duplicate on Slanetz agar (Oxoid). The chosen temperature was 15°C because it represents a bad refrigeration conditions of preservation.

Results and Discussion

The activity of the BCE was 6400 AU/ml, that means that the real concentration of bacteriocin in the sauce flasks was in fact 571 AU/ml, using the quantification method of Barefoot et al. (1983).

The evolution in the population of the indicator strain *E. faecalis* in the different flasks, are reflected in Figure 1. It must be notice that along the period of time that the experience lasts no growth ocurred in the flask with only sauce. The Figure 1 shows how in the control (sauce + *E. faecalis*) the population of the sensitive strain increase progresively, reaching a maximal population around 10^8 CFU/ml in 16 days. A positive effect was observed for the former 7 days in the sauce flask with the BCE. In this period of time a

bacteriostatic more than a bacteriocidal effect was showed. This strain has a bacteriocidal effect "in vitro" and the bacteriocidal or bacteriostatic effect "in vivo" depends on the concentration of the BCE (Rovira et al. data unpublished). After this 7 days a rapid growth of the indicator strain came about. This fact could be due to the lack of sufficient amount of bacteriocin or that some bacteriocin insensitive cells have escaped from the control. A similar evolution was showed in the sauce flask with 1% of sodium lactate. This compound has demonstrated his effectiviness as a food preservative in cooked meat products in this proportion. In this case again the effectiviness of this treatment is limited to 8 days, and after this time the population of the sensitive strain begin to increase rapidly. When a combination of BCE and 1% of sodium lactate was used the population of *E. faecalis* decrease dramatically for the former 13 days and, from this day forward, no population of the indicator strain were detected in the sauce. It seems that a combination of the bacteriocin with the organic acid has a synergic effect. In this sense, this combination could be a good way to improve the bacteriocidal or bacteriostatic power of the bacteriocin. Similar conclusions were suggested by Degnan et al. (1992) and El-Khateib et al. (1993) in wieners and in beef muscle.

Other possible way to improve the effectiviness of this kind of preservatives is the combination of different bacteriocins. Hanlin et al. (1992) showed a major bacteriocidal effect when two bacteriocins (pediocin AcH and nisin) were used in combination versus the results obtained when they were used in a single way. Kalchayanand et al. (1992) described also the effectiviness of bacteriocins pediocin AcH and nisin against Gram-negative and resistant Gram-positive bacteria with a sublethal-injury. Also, Stevens et al. (1992) demostrated the effectiviness of combining nisin with a chellating agent for inactivating Gram-negative bacteria.

All these data suggest that bacteriocins could play an important role in food preservation, especially if several of them are used in combination or together with an organic acid or acids, and as a complement of the thermal treatment and refrigeration conditions.

Conclusions

It has been demostrated that BCE plays a preservative role in a food system. This role is amplified when a mixture of bacteriocin and 1% of sodium lactate is used. In the same way, sauce seems to be a good vehicle for the bacteriocin could bring about his preservative action, since sauces are quite liquid (better diffusion), fat free often (no fat adsorbtion) and covers and surrounds the main component of the dish.

However, more detailed studies should be done in order to improve the effectiviness of bacteriocins in their use as food preservatives.

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