Use of Pediocin AcH in Meat Preservation

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Summary and Implications

Pediocin AcH shows great potential for use in the preservation of meat and meat products. The bacteriocin persisted in sterile ground meat for up to 4 days at 25°C, for 15 days at 7°C and for more than 3 months in frozen samples. The bacteriocin also remained unaffected by irradiation up to doses of 7.0 kGy and high hydrostatic pressure of up to 100 kpsi. In keeping with the 'hurdle' concept, pediocin AcH may be highly effective in controlling microbial growth in meat especially when used in conjunction with other technologies such as irradiation and high hydrostatic pressure.

Introduction

In recent years there has been an increased interest in the use of bacteriocins as all natural food preservatives and antimicrobial agents. Bacteriocins have all the characteristics that could make them one of the greatest advances in food preservation (1). Bacteriocins are biologically active proteins that have an antimicrobial activity against bacterial species related to the producer strain. Most bacteriocins have a very narrow spectrum of activity but there have been some, isolated from primarily the lactic acid bacteria group, that have a relatively broad spectrum of anti microbial activity (2,6,7). Pediocin, the bacteriocin used in this study, is produced by *Pediococcus acidilactici*, a generally recognized as safe (GRAS) organism, commonly found and used in fermented sausage production. Most pediocins are thermostable proteins and function under a wide range of pHs. Pediocin AcH has been proven to be effective against both spoilage and pathogenic organisms, including *Listeria monocytogenes, Enterococcus faecalis, Staphylococcus aureus* and *Clostridium perfringens* (3). Bacteriocins have been shown to have a greater target range and antimicrobial activity when used in conjunction with other stress inducing processes such as heating, freezing, acid treatment, chelating agents, high hydrostatic pressure and electroporation (4, 5)**.** The purpose of this study was to evaluate the applicability of bacteriocins as a preservative in meat systems in conjunction with other treatments such as high hydrostatic pressure, organic acid rinses, and irradiation. High hydrostatic pressure and irradiation are new technologies that show tremendous potential in the area of food preservation.

Materials and Methods

Culture and growth conditions. *Pediococcus acidilactici* strain H was used for the production of pediocin AcH. The strain was maintained on slants of MRS lactobacillus broth (Difco) supplemented with 2% yeast extract and 1.5% agar. *L monocytogenes* Scott A was used as an indicator to assay the pediocin AcH. It was maintained on Tryptic soy Agar (Difco) slants.

Preparation of pediocin AcH. Pediococcus acidilactici strain H was grown in TGE broth at 37°C for 16 to 18 hours. Purification of pediocin was carried out using the procedure outlined by Yang et al. (8). The culture was placed in a 75°C water bath for 15 minutes to kill the cells and proteolytic enzymes. The pH of the culture was then adjusted to 6.5 with 0.2 M sodium hydroxide to allow maximum adsorption of the pediocin onto the cell surfaces. The culture was centrifuged at 15000 x *g* for 12 minutes. The cells were washed in 5 mM sodium phosphate (pH 6.5), centrifuged again, and then suspended in 100 mM NaCl at pH 2 for maximum desorption of the bacteriocin from the cells. The suspension was centrifuged again and the supernatant used as semipurified pediocin.

Assay of Pediocin AcH. We used 0.1 ml of an 18 hr culture of *L monocytogenes* Scott A to inoculate 10 ml of Tryptic Soy broth supplemented with 0.6% yeast extract (TSBYE) and the culture was grown for 8 hours at 37°C. A lawn of 10^6 indicator cells was poured using TSBYE with 0.75% agar for both top (5 ml) and bottom (10 ml) of agar. The pediocin was serially twofold diluted and 5 µl of each dilution placed on the surface of the indicator lawn and then incubated at 37°C. The inhibitory strength was expressed in arbitrary units (AU). One AU was defined as the inverse of the highest dilution that produced an inhibition zone of diameter greater than 2 mm on the indicator lawn.

Pediocin degradation in meat. Ground beef was obtained from various retail stores and 2.5 g samples placed in irradiation bags and frozen. Then 2.5 ml of semipurified pediocin solution was added to the meat, the bags sealed and irradiated at 7 kGy with e-beam irradiation. The samples were then divided into three lots. One set of samples was stored at 25°C (room temperature), one set at 7°C (refrigeration), and one at -25°C (frozen).

In a second experiment some of the frozen samples were removed from -25°C after 24 days and stored at 7°C. Samples were removed at intervals during storage, centrifuged, and the supernatant assayed for pediocin.

Effect of Irradiation and high pressure. We placed 5 ml samples of semipurified pediocin in sterile irradiation bags and irradiated at doses of 0.5, 2.0, 3.5, 5.0 and 7.0 kGy. The samples were then assayed for pediocin activity

Five milliliter samples of semipurified pediocin were double bagged and sealed in sterile irradiation bags. They were then subjected to pressures of 30 kpsi and 100 kpsi (at room temperature). The samples were then assayed for pediocin activity.

Results and Discussion

Degradation of bacteriocin in meat. In every case 75% of the bacteriocin activity was lost within minutes of addition to the meat irrespective of the initial amount. This may be due to the binding of the bacteriocin to various meat proteins. Because the meat was irradiated at 7 kGy, and no bacterial growth occurred during storage, the drop in recoverable bacteriocin activity was ascribed to degradation by proteolytic enzymes in the meat and to a smaller extent to further binding to meat proteins. The recoverable bacteriocin activity fell to levels below 1% of that added to the meat after 4 days when stored at 25°C and after 15 days at 7°C. In frozen samples the bacteriocin levels remained high even after 3 months. The rate of loss of pediocin activity was lower in samples with larger particle size.

For frozen samples that were moved to refrigeration temperature after 24 days, the bacteriocin degradation followed a similar curve to that of samples refrigerated throughout. The bacteriocin, however, persisted longer in the samples that were previously frozen.

The bacteriocin activity remained unaffected by all levels of irradiation (up to 7 kGy) and high hydrostatic pressure (up to 100 kpsi).

Although pediocin AcH and other bacteriocins show great promise as food preservatives, study is needed in the areas of bacteriocin production, purification, and mode of action, before systems can be developed that fully use their potential.

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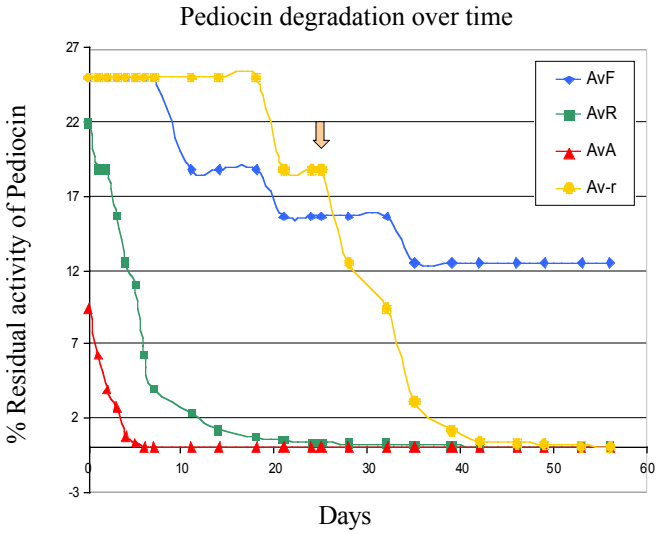

Fig.1 The arrow indicates the time of removal of samples from -25°C to 7°C. AvF, frozen samples, AvA, samples stored at room temperature, AvR, refrigerated samples, Av-r, samples stored at frozen-refrigeration temperatures