Development and characterization of an active polyethylene film containing Lactobacillus curvatus CRL705 bacteriocins

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Abstract: The development and characterization of a bacteriocin-containing polyethylene-based film is described, incorporating lactocin 705 and lactocin AL705, produced by *Lactobacillus curvatus* CRL705, and nisin. Three different procedures to obtain lactocin 705 and AL705 solution were evaluated, with the partially purified aqueous bacteriocin solution showing the highest inhibitory activity against indicator strains (*Lactobacillus plantarum* CRL691 and *Listeria innocua* 7). Pouch contact, soaking and a contact method were compared for incorporating bacteriocins onto PE-based films. Contact between the PE film and bacteriocin solution was the most effective, resulting in a more uniform distribution of bacteriocins on the film surface and using less active solution. The minimal inhibitory concentration of bacteriocin solution was 267 AU cm^{-3} (lactocin 705) and 2133 AU cm^{-3} (lactocin AL705), while the minimal contact time was 1 h. When relative inhibition area for antilisterial activity of the active films was compared, those treated with *L. curvatus* CRL705 bacteriocins displayed higher inhibitory activity than nisin-treated films. Functional properties of active PE-films containing lactocin 705 and AL705 showed no differences compared with non-active control films. Bacteriocin-active PE-based films are shown to be highly effective in inhibiting growth of *Listeria*. The potential use of commercially available packaging films as bacteriocins carriers may benefit active-packaging systems.

Keywords: food packaging; active packaging; bacteriocins; antilisterial activity

Abbreviations: LAB, lactic acid bacteria; YE, yeast extract; PE, polyethylene; PPABs, partially purified aqueous bacteriocin solutions, containing *L. curvatus* CRL705 bacteriocins and obtained by method (i); PPIBs, partially purified isopropanol bacteriocin solutions, containing *L. curvatus* CRL705 bacteriocins and obtained by method (ii); SPEBs, solid-phase extraction bacteriocin solutions, containing *L. curvatus* CRL705 bacteriocins and obtained by method (iii); MIC, minimal inhibitory concentration; MCT, minimal contact time; WVTR, water vapour transmission rate.

Introduction

Active packaging has been introduced as an innovative food-packaging concept in response to the continuous changes in consumer demands and market trends. Active packaging performs some

important roles other than providing an inert barrier between the product and external environment (Church and Parsons 1995). The incorporation of antimicrobial agents directly into polymeric packaging allows the industry to combine the preservative function of antimicrobials with the protective function of packaging. Active packaging technologies are designed to extend shelf-life, while maintaining the nutritional quality and safety of foods, and involve interactions between the food, the packaging material and the internal gaseous atmosphere (Labuza 1990).

In recent years, attention has focused on the prevention of initial adhesion of microbial contaminants by the application of an antimicrobial substance to the surface rather than trying to remove the undesirable bacteria once they have adhered. Unfortunately, some of the traditional methods used for food preservation (thermal processing, drying, freezing, refrigeration, irradiation, modified atmosphere packaging, and adding antimicrobial agents or salts) cannot be applied to foods such as fresh meats and ready-to-eat (RTE) products. Direct surface application of antibacterial substances onto foods (sprays or dip solutions) had limited benefits since the active substances can become neutralized on contact or diffuse rapidly from the surface to the bulk food (Quintavalla and Vicini 2002). On the other hand, incorporation of antimicrobial agents into food formulations may result in partial inactivation of the active substances by food constituents and is, therefore, expected to have only a limited effect on surface microflora.

The literature provides evidence that antimicrobial substances may be effective as indirect food additives incorporated into food packaging materials, for the reduction in surface contamination of processed foods (Vermeiren et al. 2002; Cooksey 2005; Joerger 2007).

Various types of active substances can be incorporated into the packaging material to improve its functionality and give it new or extra functions. A range of antimicrobials have been incorporated into or coated onto different film types, such as bacteriocins, acids and their salts and anhydrides, plant extracts, enzymes, triclosan and antifungal agents. Even though it is difficult to distinguish between the technical function of an additive as solely a packaging antimicrobial without any impact on the food itself, the regulatory status of packaging in the European Union permits the use of antimicrobial additives (Directive 2002/72/EC and its amendments). Among the bacteriocins used as antimicrobials, nisin produced by *Lactococcus lactis* has GRAS status and has been approved as a food additive by both the US FDA and WHO. It is also the antimicrobial most commonly incorporated into films, either as singly or combined with other antimicrobials (Joerger 2007). Since the target of these antimicrobial peptides are Gram positive organisms, they have been applied to natural as well as synthetic materials as an effective approach to reduce *Listeria monocytogenes* in foods. For meat and meat products in particular, different antilisterial packaging materials and coatings have been evaluated using nisin (Ming et al. 1997; Siragusa et al. 1999; Scannell et al. 2000; Franklin et al. 2004; Grower et al. 2004a; Guerra et al. 2005), pediocin (Nielsen et al. 1990; Ming et al. 1997) and an antilisterial bacteriocin produced by *Lactobacillus curvatus* 32Y (Mauriello et al. 2004). Despite intensive research and development and increasing commercialization of active and intelligent packaging systems, no specific method exist in national and international legislation to determine their suitability in direct contact with foods. The result is that the legislation applying to traditional packaging materials has been applied to active and intelligent packaging systems (Robertson 2006).

Lactocin 705 and lactocin AL705, two bacteriocins produced by *Lactobacillus curvatus* CRL705 of meat origin, a two-component (class IIb) and an antiListeria (class IIa) compound, respectively, have

been characterized (Castellano et al. 2003, 2004; Cuozzo et al. 2003). *Lactobacillus curvatus* CRL705 was used as a protective culture in fresh beef and shown to be effective against *Listeria innocua* and *Brochothrix thermosphacta*, as well as the indigenous lactic acid bacteria (LAB) contaminants, with lactocin 705 and AL705 being inhibitory under vacuum-packaging conditions and low temperatures (Castellano et al. 2004; Castellano and Vignolo 2006). The overall objective of this work was to develop and characterize a PE-based packaging film containing the bacteriocins, lactocin 705 and lactocin AL705, both produced by *L. curvatus* CRL705, for its potential use in meats and RTE products.

Materials and methods

Bacterial strains and growth conditions

Lactobacillus curvatus CRL705 (producer of lactocin 705 and lactocin AL705) and *Lactobacillus plantarum* CRL691 (an indicator of lactocin 705), isolated from dry-fermented sausages and in the CERELA culture collection, were grown in MRS broth (Britania, Buenos Aires, Argentina) at 30°C. *Listeria innocua* 7 (an indicator of lactocin AL705) was obtained from the Unité de Recherches Laitières et Génétique Appliquée, INRA (France) and was grown in trypticase soy broth (TSB) (BBL, Cockeysville, MD, USA) with 5 mg cm^{-3} of added yeast extract (YE) at 30°C. All strains were maintained and stored at 20°C in 0.15 g cm^{-3} glycerol.

Bacteriocin preparation

Lactocin 705 and lactocin AL705 were obtained by growing *L. curvatus* CRL705 in MRS broth at 30° C for 18 h. The culture was centrifuged (2500 g for 15 min), the supernatant precipitated using 0.44 g cm^{-3} ammonium sulphate, centrifuged (20,000 g for 20 min) and freeze-dried. We evaluated various bacteriocin solutions:

(i) Aqueous bacteriocin solution: the active powder was re-suspended in water and used as a partially purified aqueous bacteriocins solution (PPABs);

(ii) Isopropanol extraction: NaCl was added to the aqueous bacteriocin solution to saturation and the solution extracted three times with ¼ volume of isopropanol; the organic phases were then pooled (PPIRs);

(iii) Solid-phase extraction: the modified method of Berjeaud and Cenatiempo (2004) was used, in which the aqueous bacteriocin solution was applied to a SPE-C_{18} cartridge (Varian), washed successively with 10 and 30% isopropanol +20 mM ammonium acetate and the bacteriocins eluted using four bed volumes of 40% isopropanol +20 mM ammonium acetate (SPEBs). A stock nisin solution was prepared by dissolving 100 mg of Nisaplin® (Danisco Argentina SA, Buenos Aires, Argentina) in 0.02 N HCl to assure complete solubilization. Finally, 1 N NaOH was added until pH 2.5, and distilled water to give a final volume of 10 cm^3 and a nisin concentration of 10 mg cm^{-3}. A solution of 1 mg cm^{-3} was prepared from 10 mg cm^{-3} solution.

Bacteriocins activity quantification

Activity of the bacteriocin solutions, expressed in arbitrary units (AU) per cm^3 , was determined by a modification of the agar diffusion and critical dilution assay described by Pongtharangkul et al. (2004).

Briefly, 15 µl of serial twofold dilutions of the bacteriocin solution was added to 5 mm diameter wells cut in semisolid MRS and TSA + 5 mg cm^{-3} YE seeded with *L. plantarum* CRL691 and *L. innocua* 7, respectively, as indicator organisms. The plates were stored at 4°C for 24 h to allow pre-diffusion, incubated for 16–18 h at 30°C and examined for zones of inhibition. The titer was defined as the reciprocal of the highest dilution yielding a visible zone of inhibition on the indicator lawn. Each determination was performed in duplicate.

Preparation of active film and activity determination

T6040B linear low-density polyethylene (PE)-based film with a barrier layer of ethylene vinyl alcohol copolymer (Cryovac; Sealed Air Co., Buenos Aires, Argentina) was used as the packaging material. For the adsorption of bacteriocins on the plastic film, three methods were evaluated using PPABs (see above) containing lactocin 705 (4267 AU cm^{-3}) and lactocin AL705 (8533 AU cm^{-3}).

(i) Pouch-contact: pouches made of T6040B film were prepared and thermo-sealed at 150°C for 1 s and 2.5 Pa using a thermo-sealing machine (TP-701S Heat Seal Tester, Tokyo, Japan). The active solution (46.9 cm^3) was placed inside the pouch, clip-closed and shaken gently for 24 h.

(ii) Soaking: T6040B films were soaked in 1.5 cm^3 of PPABs for 24 h.

(iii) Contact: T6040B films were placed in contacted with 4.6 cm^3 of the PPABs active solution during 24 h.

The area/volume ratio in each method was 4.26, 3.00 and 4.26, respectively. After each treatment, the bacteriocin solution was removed, the plastic rinsed with sterile distilled water and dried for 10 min at 50°C. Antimicrobial activity of impregnated and control plastic films was determined by placing 1.0 cm diameter punched circles directly on the semisolid agar plates seeded with the sensitive organisms. Film bioactivity was evidenced as an inhibition zone of the indicator organisms beneath and around the packaging material and was expressed as relative inhibition area (inhibition zone around packaging film/film area). The diameter of the inhibition zone around each film was measured using a caliper. Samples were run in triplicate.

MIC and MCT determination

MIC and MCT of PPABs necessary to reach the adsorption equilibrium of the bacteriocins onto the film surface were determined using three concentrations of PPABs (0.1, 1 and 10 mg cm^{-3}). The films were placed in contact with the active solutions for 5, 20, 60, 480 and 1440 min. Nisin solutions (1 and 10 mg cm^{-3}) were also placed in contact with PE films for 1 h. Triplicate samples were run simultaneously.

Functional properties of the active film

Tensile strength and elongation of treated and non-treated films were compared using an Instron model 1125 according to ASTM D 638-07 with a grips separation of 65 mm and test speed 500 mm min^{-1}. The water vapour transmission rate (WVTR) of the films was evaluated accordingly ASTM E 96/E 96 M-05. The assayed PE film was used to seal an aluminum cup containing anhydrous silica desiccant (0.048 m^2 exchange film area) and placed in a controlled humidity and temperature (50% RH and 25°C) room. The WVTR (g H_2O m^{-2} day^{-1}) was determined by the increase in cup weight over time at the mass transfer steady-state. Overall migration into the aqueous food simulant was carried out according to European Prestandards ENV 1186–7 at 40°C during 10 days. All tests were conducted in triplicate.

Results

The antimicrobial substances produced by *L. curvatus* CRL705 were partially purified from the culture supernatant and used as a source of bacteriocins for the active PE-based film. When antimicrobial activity of the bacteriocin solutions containing lactocin 705 and lactocin AL705 was compared, PPABs and PPIBs showed higher titers than SPEBs (4267, 2133 and 533 AU cm^{-3} for lactocin 705 and 8533, 2133 and 1067 AU cm^{-3} for lactocin AL705, respectively). In addition, PPABs exhibited a higher inhibitory activity than PPIBs against *L. plantarum* CRL691 and *L. innocua* as indicator microorganisms. Films containing *L. curvatus* CRL705 bacteriocins were obtained by pouch-contact, soaking and contact. Results showed that soaking was more effective than the pouch-contact method as a larger inhibition zone and more uniform distribution of bacteriocins on the film surface was observed for both lactocin 705 or AL705 (Figure 1a and b).

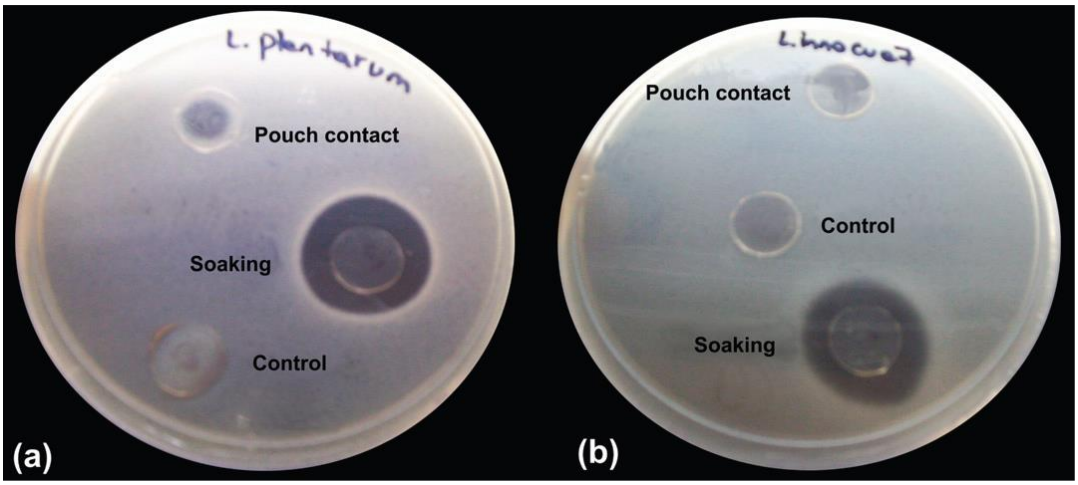

Figure 1. Antimicrobial activity of PE-based films treated with PPABs using different methods against (a) *L. plantarum* CRL691 and (b) *L. innocua* 7.

When relative inhibition areas of the films obtained by soaking and contact were compared (Figure 2), no significant difference was observed ($p>0.05$) but a lesser volume of active solution was required with the contact method.

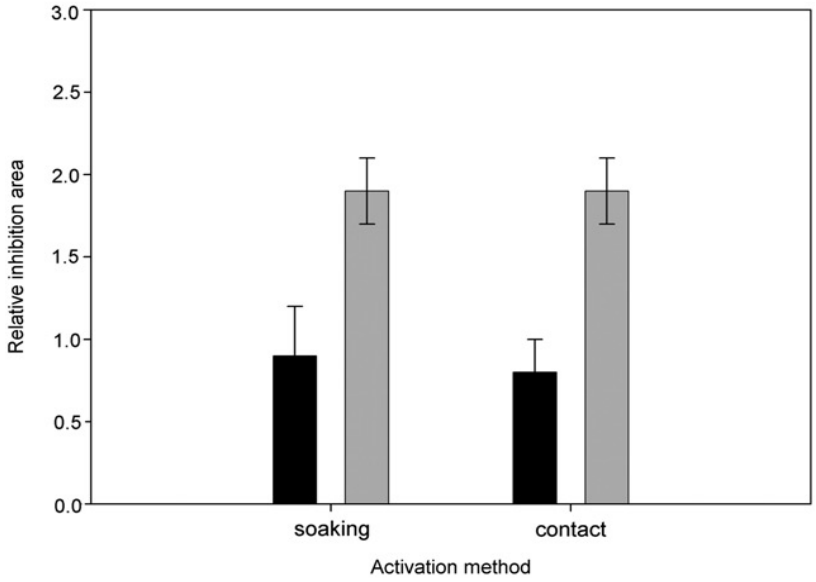

Figure 2. Relative inhibition areas of antimicrobial PE-based films developed by soaking and contact method assayed against (■) *L. plantarum* CRL691 and (■) *L. innocua* 7.

Three different PPAB concentrations and contact times were assayed to determine the MIC of the contact solution plus the MCT. A relative inhibition area 41.0 was obtained after a 1 h contact when PPABs at a concentration of 1 and 10 mg cm^{-3} (corresponding to 2133 and 12800 AU cm^{-3} of lactocin AL705) was applied. On the other hand, no antimicrobial activity was observed on films surface treated with a 400 AU cm^{-3} solution (0.1 mg cm^{-3} PPABs) against *L. innocua* 7 (Figure 3).

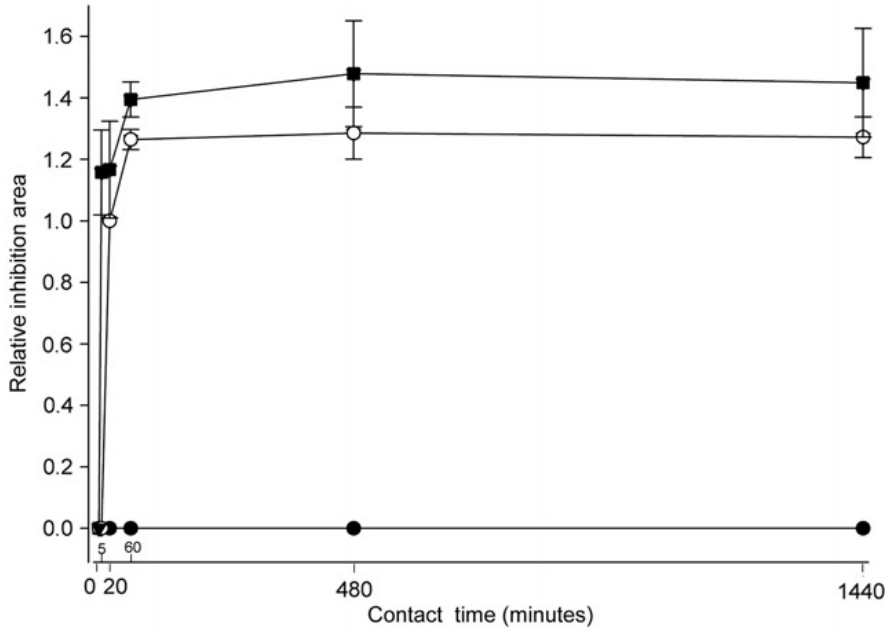

Figure 3. Effect of lactocin AL705 activity and contact time on relative inhibition areas of PE-based films against *L. innocua* 7. (●) PPABs, 0.1 mg cm^{-3} (400 AU cm^{-3}), (○) PPABs, 1mg cm^{-3} (2133 AU cm^{-3}) and (■) PPABs, 10 mg cm^{-3} (12800 AU cm^{-3}).

When the active film was assayed against *L. plantarum* CRL691, the MIC for lactocin 705 was 267 AU cm^{-3} (PPABs, 1 mg cm^{-3}) after a 1 h contact; no activity was observed using a 0.1 mg cm^{-3} PPABs solution (67 AU cm^{-3}) (Figure 4).

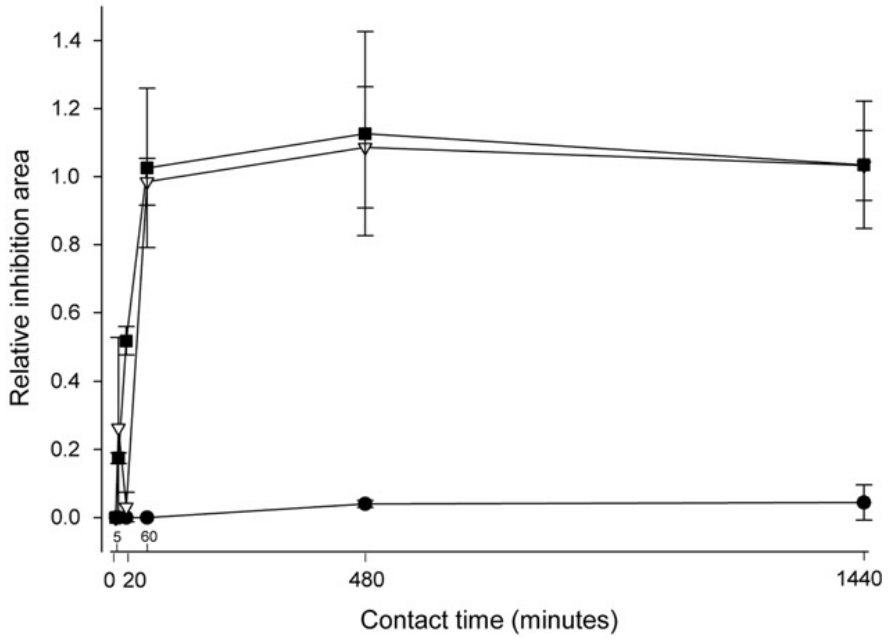

Figure 4. Effect of lactocin 705 activity and contact time on relative inhibition areas of PE-based films against *L. plantarum* CRL691. (●) PPABs, 0.1 mg cm^{-3} (67 AU cm^{-3}), (▽) PPABs, 1mg cm^{-3} (267 AU cm^{-3}) and (■) PPABs, 10 mg cm^{-3} (4267 AU cm^{-3}).

Antimicrobial activities of PE films treated with PPABs (1 mg cm^{-3}) and nisin against both *L. plantarum* CRL691 and *L. innocua* 7 were compared. Nisin-treated films (1 and 10 mg cm^{-3}) displayed large inhibition zones when *L. plantarum* CRL691 was used as indicator organism, but failed to inhibit *L. innocua* 7 on the agar plates (Figure 5).

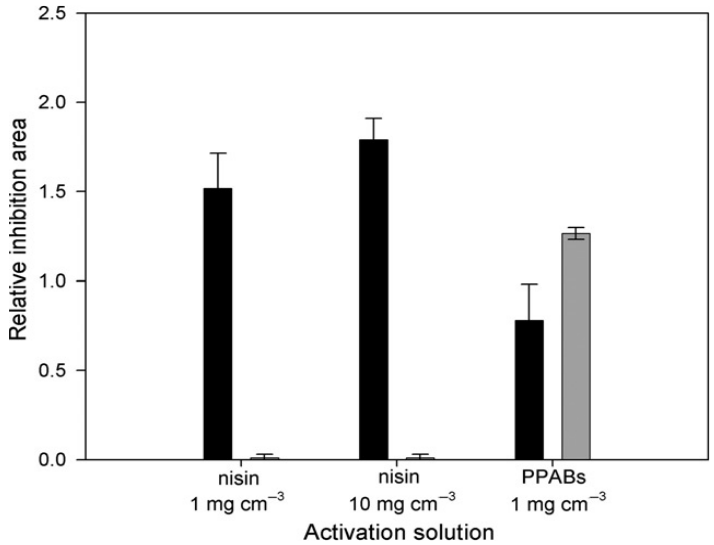

Figure 5. Relative inhibition areas of PE-based films treated with nisin (1 and 10 mg cm^{-3}) and PPABs (1 mg cm^{-3}) against (■) *L. plantarum* CRL691 and (■) *L. innocua* 7.

In addition, even when PE films treated with PPABs (1 mg cm^{-3}) exhibited a lower relative inhibition area against *L. plantarum* CRL691 than nisin, they were more effective in inhibiting *L. innocua* 7. When functional properties of active and control films were evaluated, the values obtained for tensile strength (49±3 vs. 48±3 MPa), elongation (377±19 vs. 372±13%) and WVTR (0.55±0.08 vs. 0.55±0.03 g day^{-1} m^{-2}), respectively, showed no significant differences ($p>0.05$). Moreover, when an aqueous simulant was used, overall migration values were lower than 1 mg dm^{-2} for both active and non- active films.

Discussion

In this study, conditions for the development of active PE-based films using the bacteriocins, lactocin 705 and lactocin AL705, produced by *Lactobacillus curvatus* CRL705 were evaluated. Results clearly demonstrated a retention of antimicrobial activity when adsorbed onto PE-based films. The various procedures used to obtain bacteriocin solutions from *L. curvatus* CRL705 showed that PPABs and PPIBs displayed higher antimicrobial activity than SPEBs, in agreement with dilutions carried out with SPE-C_{18} cartridges. Moreover, since PPABs was easier to obtain and its activity was higher than PPIBs, it was selected as the active solution.

Three methods of binding bacteriocins from *L. curvatus* CRL705 onto PE-based films were evaluated. Soaking and a contact procedure were demonstrated to be more effective than pouch-contact. Clean, homogeneous and confined inhibition areas were observed, suggesting that the bacteriocins were uniformly bound to the surface of the film and diffused regularly into the agar. Soaking was also applied to develop active PE-oriented polyamide (OPA) films with an antilisterial bacteriocin from *Lactobacillus curvatus* 32Y (Mauriello et al. 2004), in which non-uniform bacteriocin diffusion from the film into the agar was obtained. Since only attached bacteriocins could exert antimicrobial activity, the washing step of the active PE-based film after soaking may be responsible for the uniform diffusion of lactocin 705 and lactocin AL705 into the agar. Several studies have reported the efficacy of the contact method in providing active films incorporating antimicrobial substances. Nisaplin® was incorporated into cellophane-based packaging by contact, which was effective for the control of microbial growth in chopped meat (Guerra et al. 2005). In addition, Daeschel et al. (1992) reported the antimicrobial activity of nisin after adsorption onto hydrophobic and hydrophilic silicon surfaces using the contact method. Although the contact and soaking methods yielded similar results in our study, less bacteriocin solution was required with the contact method; therefore, this method was chosen for obtaining active PE-based films. Bacteriocins other than nisin were also applied by various methods to develop active plastic materials for L. monocytogenes inhibition in agar media or food systems, such as the bacteriocin from *L. curvatus* 32Y and CWBI-B28 (Mauriello et al. 2004; Ercolini et al. 2006; Ghalfi et al. 2006), lacticin 3147 produced by *Lactococcus lactis* subsp. *lactis* (Scannell et al. 2000) and pediocin from *Pediococcus acidilactici* (Ming et al. 1997).

The MIC of the antibacterial solution, defined as the lowest concentration of bacteriocin yielding films that result in complete inhibition of visible growth beneath and around the active film, corresponded to PPABs with a concentration of 1 mg cm^{-3} (2133 AU cm^{-3} for lactocin AL705 and 267 AU cm^{-3} for lactocin 705). An adsorption plateau was attained after 1 h of contact with the active solution, indicating saturation of both bacteriocins in the PE film after this contact period. In other

words, an antimicrobial PE-based film was developed using the PPABs (1 mg cm^{-3}) containing lactocin AL705 and lactocin 705 from *L. curvatus* CRL705 after 1 h of contact time. Due to the fact that nisin is the only bacteriocin approved for food applications, we used it in this study for comparative purposes. In this study, the lack of sensitivity of *L. innocua* to nisin-active films compared to *L. plantarum* CRL691 (Figure 5) is in agreement with Cutter and Siragusa (1994, 1996) and Cutter et al. (2001), who found *Brochothrix thermosphacta* to be more sensitive to nisin than *L. innocua*. Moreover, using well-diffusion assays, these authors also found that *L. innocua* was more resistant to nisin than *L. monocytogenes* Scott A. On the other hand, sensitivity evaluations of various *Listeria* strains to lactocin AL705, enterocin CRL35 and nisin indicated that the non-nisin antilisterial bacteriocins were more inhibitory than nisin – their susceptibility to bacteriocins being strain-dependent (Castellano et al. 2001).

To establish the effect of bacteriocin solutions on the functional properties of PE-based film, they were compared with those of the developed active films. When choosing a packaging film for any application, it is important to select the film on the basis of product requirements (Grower et al. 2004a). No differences were found between the active and control films when mechanical properties and WVTR were evaluated. Grower et al. (2004a) reported variations in tensile strength and moisture barrier properties of a low density polyethylene film coated with a cellulose-based solution incorporating nisin with increasing nisin concentration. In a similar study, the release of nisin from a coating was found to be uncontrolled, with *L. monocytogenes* inhibition being inconsistent (Grower et al. 2004b). These variations in mechanical properties may be ascribed to the coating methodology used for the incorporation of antimicrobial substances onto the film. For example, the performance of the contact-obtained films in this study showed no differences compared to non-treated films. Overall migration limit is an estimate of the inertness of a plastic material to prevent unacceptable changes in the composition of foodstuffs (Czerniawski and Pogorzelska, 1997). Our results showed overall migration values below the required limit specified in EU Directive 02/72/EC (2002). In conclusion, the potential for incorporating antimicrobial peptides from LAB onto PE-based plastic materials has been demonstrated. The active PE film obtained by ‘‘contact’’ with lactocin 705 + lactocin AL705, produced by *L. curvatus* CRL705, proved to be more effective than nisin in inhibiting the growth of *Listeria*, without changing the functional properties of the film.

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References

ASTM E 96/E 96 M-05. 2005. Standard test methods for water vapor transmission of materials. Philadelphia, PA: American Society for Testing and Materials.

ASTM D 638-07. 2007. Standard test method for tensile properties of plastics. Philadelphia, PA: American Society for Testing and Materials.

Berjeaud J-M, Cenatiempo Y. 2004. Purification of antilisterial bacteriocins. In: Spencer JFT, Ragout de Spencer AL, editors. Methods in molecular biology. Totowa, NJ: Humana Press. p. 225–233, Chapter 21.

BS EN 1186–7 2002, Materials and articles in contact with foodstuffs. Plastics. Part 7: Test methods for overall migration into aqueous food simulants using a pouch. London: British Standards Institute.

Castellano P, Vignolo G. 2006. Inhibition of Listeria innocua and Brochothrix thermosphacta in vacuum-packaged meat by the addition of bacteriocinogenic Lactobacillus curvatus CRL705 and its bacteriocins. Lett Appl Microbiol. 43:194–199.

Castellano P, Farí as ME, Holzapfel W, Vignolo G. 2001. Sensitivity variations of Listeria strains to the bacteriocins lactocina 705, enterocin CRL35 and nisin. Biotechnol Lett. 23:605–608.

Castellano P, Raya R, Vignolo G. 2003. Mode of action of lactocin 705, a two-component bacteriocin from Lactobacillus casei CRL705. Int J Food Microbiol. 85:35–43.

Castellano P, Holzapfel W, Vignolo G. 2004. The control of Listeria innocua and Lactobacillus sakei in broth and meat slurry with the bacteriocinogenic strain Lactobacillus casei CRL705. Food Microbiol. 21:291–298.

Church IJ, Parsons AL. 1995. Modified atmosphere packaging technology: A review. J Sci Food Agric. 67:143–152.

Cooksey K. 2005. Effectiveness of antimicrobial food packaging materials. Food Addit Contam. 22:980–987.

Cuozzo S, Castellano P, Sesma F, Vignolo G, Raya R. 2003. Differential roles of the two-component peptides of Lactocin 705 in antimicrobial activity. Curr Microbiol. 46:180–183.

Cutter CN, Siragusa GR. 1994. Decontamination of beef carcass tissue with nisin using a pilot scale model carcass washer. Food Microbiol. 11:481–489.

Cutter CN, Siragusa GR. 1996. Reductions of Listeria innocua and Brochothrix thermosphacta on beef following nisin spray treatments and vacuum packaging. Food Microbiol. 13:23–33.

Cutter CN, Willett JL, Siragusa GR. 2001. Improved antimicrobial activity of nisin-incorporated polymer films by formulation change and addition of food grade chelator. Lett Appl Microbiol. 33:325–328.

Czerniawski B, Pogorzelska Z. 1997. Investigations on overall migration of various plastic materials and articles used in contact with foodstuffs. Packag Technol Sci. 10:261–270.

Daeschel MA, McGuire JM, Al-Makhlafi H. 1992. Antimicrobial activity of nisin adsorbed to hydrophilic and hydrophobic silicon surfaces. J Food Protect. 55:731–735.

EC, 2002, Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. Off J the Eur Commun. L220: 18–58.

Ercolini D, La Storia A, Villani F, Mauriello G. 2006. Effect of a bacteriocin-activated polythene film on Listeria monocytogenes as evaluated by viable staining and epifluorescence microscopy. J Appl Microbiol. 100:765–772.

Franklin NB, Cooksey KD, Getty KJ. 2004. Inhibition of Listeria monocytogenes on the surface of individually packaged hot dogs with a packaging film coating containing nisin. J Food Protect. 67:480–485.

Ghalfi H, Allaoui A, Destain J, Benkerroum N, Thonart P. 2006. Bacteriocin activity by Lactobacillus curvatus CWBI-B28 to inactivate Listeria monocytogenes in cold-smoked salmon during 4°C storage. J Food Protect. 69:1066–1071.

Grower JL, Cooksey K, Getty KJ. 2004a. Development and characterization of an antimicrobial packaging film containing nisin for inhibition of Listeria monocytogenes. J Food Protect. 67:475–479.

Grower JL, Cooksey K, Getty KJ. 2004b. Release of nisin from methylcellulose-hydroxypropyl methylcellulose film formed on low-density polyethylene film. J Food Sci. 69:107–111.

Guerra NP, Mací as CL, Agrasar AT, Castro LP. 2005. Development of bioactive packaging cellophane using Nisaplin® as biopreservative agent. Lett Appl Microbiol. 40:106–110.

Joerger RD. 2007. Antibacterial films for food applications: A quantitative analysis of their effectiveness. Packag Technol Sci. 20:231–273.

Labuza TP. 1990. Active food packaging technologies. Food Sci Technol Today. 4:53–56.

Mauriello G, Ercolini D, La Storia A, Casaburi A, Villani F. 2004. Development of polythene films for food packaging activated with an antilisterial bacteriocin from Lactobacillus curvatus 32Y. J Appl Microbiol. 97:314–322.

Ming X, Weber GH, Ayres JW, Sandine WE. 1997. Bacteriocins applied to food packaging materials to inhibit Listeria monocytogenes on meats. J Food Sci. 62:413–415.

Nielsen JW, Dickson JS, Crouse JD. 1990. Use of a bacteriocin produced by Pediococcus acidilactici to inhibit Listeria monocytogenes associated with fresh meat. Appl Environ Microbiol. 56:2142–2145.

Pongtharangkul T, Demirci A. 2004. Evaluation of agar diffusion bioassay for nisin quantification. Appl Microbiol Biotechnol. 65:268–272.

Quintavalla S, Vicini L. 2002. Antimicrobial food packaging in meat industry. Meat Sci. 62:373–380.
Robertson GL. 2006. Active and intelligent packaging. In: Robertson GL, editor. Food packaging principles and practice. 2nd ed. New York: Marcel Dekker. p. 286–311, Chapter 14.

Scannell AG, Hill C, Ross RP, Marx S, Hartmeier W, Arendt EK. 2000. Development of bioactive food packaging materials using immobilized bacteriocins lacticin 3147 and Nisaplin. Int J Food Microbiol. 60:241–249.

Siragusa GR, Cutter CN, Willett JL. 1999. Incorporation of bacteriocin in plastic retains activity and inhibits surface growth of bacteria on meat. Food Microbiol. 16:229–23.

Vermeiren L, Devlieghere F, Debevere J. 2002. Effectiveness of some recent antimicrobial packaging concepts. Food Addit Contam. 19:163–171.