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Antibacterial efficacy of nisin, bacteriophage P100 and sodium lactate against *Listeria monocytogenes* in ready-to-eat sliced pork ham

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ABSTRACT

The effectiveness of bacteriophage P100, nisin and sodium lactate, individually and in combination, in inhibiting *Listeria monocytogenes* in ready-to-eat pork ham slices was assessed. The antimicrobials were applied to the surfaces of ready-to-eat pork ham slices, which were inoculated with a mixture of *L. monocytogenes*. Among the individual antimicrobial treatments, bacteriophage P100 was the most effective, decreasing *L. monocytogenes* to undetectable levels at zero and 72 h post-infection. Sodium lactate was the least effective treatment. Treatment with nisin at zero h significantly reduced initial cell density ($p < 0.05$). However, this pattern was not observed at 72 h of storage. A significant difference ($p < 0.05$) existed between the results of separate bacteriophage and nisin treatments after refrigerated storage, but not immediately upon inoculation of the bacteria. The results showed that the use of bacteriophage P100 is the method of choice for the control of bacteria.

Introduction

The first conclusive connection of *Listeria monocytogenes* with a foodborne outbreak occurred in 1981, stimulating researchers to determine the ubiquity of the organism and its mode of transmission. Since then, when the mortality rate did not seem to diminish over years, *L. monocytogenes* has gained recognition as an important foodborne pathogen.[1]

Although *L. monocytogenes* is inactivated by thermal treatments in processed food, post-processing cross-contamination from equipment and the environment may occur due to the pathogen's persistence and ability to form biofilms.[2,3]

Considering that *L. monocytogenes* is capable of growing at refrigerated temperatures, antimicrobial strategies to overcome this microorganism's tolerance for low temperatures are essential, and the food industry has sought more effective methods to control this pathogen.[4]

Despite advances in hurdle technology, food preservation techniques are still evolving, not only in developing countries but also in the industrialized world. The concept of hurdle technology has been applied in the food industry following the observations that the rate of microorganism survival

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decreases greatly when they are confronted with multiple antimicrobial factors, or hurdles.[5]

Currently, the application of bacteriocins as part of hurdle technology has gained attention. Bacteriocins are antimicrobial active peptides or proteins synthesized in the ribosomes of bacterial cells and secreted by some bacteria against microorganisms that are closely related to the producer organism.[6] They are bacteriostatic or bactericidal against Gram-positive bacteria,[7–10] but bacteriocins synthesized by Gram negative bacteria (e.g. colicins) are active against Gram negative bacteria. These antimicrobial peptides have been traditionally used as bio-preservatives to extend the shelf lives of food products without compromising their nutritional and organoleptic properties[7,11,12] and they have already been successfully applied in several food systems to control the growth of *L. monocytogenes*.[13,14]

Several bacteriocins show synergism when used in combination with other antimicrobials, including chemical preservatives, phenolic compounds, and other natural antimicrobial proteins.[15,16] The effectiveness of bacteriocins is often dependent upon environmental factors such as pH and temperature, interaction with food components, precipitation, inactivation, or uneven distribution of bacteriocin in the food matrix, and food microbiota.[17]

Nisin, the bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, has been successfully used as an antibacterial agent in various food products.[8,16] Currently, nisin is the only bacteriocin widely used as a food preservative, and it has been accepted by the World Health Organization as a food bio-preservative.[6,15] However, its successful application in meat systems has been limited due its interaction with phospholipids, low solubility and inactivation by endogenous meat enzymes.[15]

Another strategy to ensure food safety is the use of bacteriophages, which are viruses that infect and kill bacteria. Phages are components of the natural microflora that are present throughout food production, from the farm to the retail outlet.[17] They are stable and recovered from soil, sewage, water, farm and processing plant effluents, feces, and retail foods.[17,18] The USFDA has approved a bacteriophage preparation made from six individually purified bacteriophages for use as an antimicrobial agent against *L. monocytogenes* on ready-to-eat meat and poultry products.[19] The commercial product named LISTEX P100 was approved as a food bio-preservative and granted GRAS (Generally Recognized as Safe) status.[20]

Organic acids with short chains and/or their salts are frequently used as chemical decontaminants and have also been granted GRAS status.[21,22] Organic acids cross the cell membrane in their undissociated form and dissociate in the cytoplasm, causing a decrease in intracellular pH, which significantly impacts cell metabolism, resulting in reduced growth.[23,24] They are extensively used in the meat and poultry industries to enhance antimicrobial benefits.[25–27]

The antimicrobial effects of the salts of organic acids, either alone or in combination with other food additives, on the survival and growth of *L. monocytogenes* has been examined and reported[4,27–30]; however, little is known about their effects in combination with other antimicrobial substances. The growth of *L. monocytogenes* in solutions containing sodium lactate depends mainly on a product's water content, storage temperature and, to a lesser extent, the amount of nitrite that it contains.[29]

The present study was undertaken to evaluate the inhibitory effect of nisin, alone and in combination with sodium lactate and bacteriophage P100, on a mixture of two strains of *L. monocytogenes* in artificially inoculated ready-to-eat sliced pork ham both immediately and after three days under refrigerated temperature.

Materials and methods

Pork ham product: Approximately 500 g of ready-to-eat pork ham slices were collected at a local supermarket of Salvador, BA, Brazil, and transported in iced containers to the laboratory for analysis. The product was sliced, weighed and packaged immediately before purchased (three days of shelf life).

Nisin: Nisin from *L. lactis* was purchased from Sigma–Aldrich (Sigma-Aldrich Brasil Ltda, São Paulo, SP, Brazil, ref. n° 5764). A stock solution was prepared with 0.1 g of nisin and 10 mL of 0.02 M HCl filter-sterilized through a 0.22-μm membrane (Millipore). Before each experiment, nisin was diluted with 10 mM phosphate buffer (pH 6.4) to 50 μg/L.[10]

Sodium lactate: Commercial sodium DL-lactate (50% purity, U.S.P.) was obtained from Synth (Synth Brasil Ltda, São Paulo, SP, Brazil). A stock solution at 2% (v/w) was prepared and sterilized at 121 °C for 15 min.

Bacteriophage P100: LISTEX™ was purchased from EBI Food Safety (Nieuwe Kanaal 7P, 6709 PA Wageningen, The Netherlands). Before use, the titer of phage P100 was determined according to a protocol suggested by EBI Food Safety (personal communication).

Bacteria, phage and growth conditions

L. monocytogenes, serotype 1/2a (B7, AL48/15, institutional strain collection) isolated from ready-to-eat turkey breast slices in a previous study in our laboratory and *L. monocytogenes* strain Scott A – ATCC 15313 (serotype 4b) were used to inoculate samples of ready-to-eat pork ham slices. *L. ivanovii* WSLC 3009 (SLCC 4769, institutional strain collection) was used as a helper strain to determine the titer of the P100 bacteriophage (LISTEX™ P100), provided by EBI Food Safety.

The cultures of *L. monocytogenes* and *L. ivanovii* were stored in Hogness medium (1.3 mM $K_2HPO_4 \cdot 3H_2O$, 1.3 mM KH_2PO_4, 2.0 mM citrate-Na·$2H_2O$, 1.0 mM $MgSO_4 \cdot 7H_2O$, and 4.4% (v/v) glycerol) and frozen at −80 °C.[32]

Before use, the *L. monocytogenes* cultures were activated separately in tryptic soy broth (Himedia, São Paulo, SP, Brazil) supplemented with 0.6% (w/v) of yeast extract (Himedia) (TSB-YE) at 35 °C overnight in a shaker (Cientec model CT 712, São Paulo, SP, Brazil) at 150 rev/min. *L. ivanovii* culture was grown overnight at 30 °C in a half-concentrated brain-heart infusion broth (BHI 1/2 (v/v), Difco Code No. 237500) with the NaCl concentration adjusted to 5 g/L. Following the incubation, the cultures were centrifuged at 3600 × *g* for 5 min in a microcentrifuge (Digital Spectrafuge 24D, Model C2400-24D,

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