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Study of the *Lactobacillus sakei* protective effect towards spoilage bacteria in vacuum packed cooked ham analyzed by PCR–DGGE

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Abstract

The effectiveness of *Lactobacillus sakei* B-2 inoculated as a protective culture on the inhibition of spoilage bacteria on sliced vacuum packed cooked ham was investigated by using culture-dependent and -independent approaches. Total microbial DNA was directly extracted from both control and treatment samples, and subjected to a nested PCR protocol, PCR–DGGE analysis was used to identify and monitor the dynamic changes in the microbial population, followed by partial 16S rDNA sequencing. The DGGE profile demonstrated that the protective culture effectively suppressed growth of predominant spoilage bacteria *L. sakei*, *Lactobacillus curvatus* and *Leuconostoc mesenteroides* in cooked ham during storage at 4 °C, however, growth of uncultured *Leuconostoc* was not inhibited. The shelf-life of this product inoculated with *L. sakei* B-2, at levels of 5.91 ± 0.04 $\log_{10}$ CFU g^{-1} was 35 days, compared to 15 days of control samples, when the ham was stored at 4 °C.

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1. Introduction

Sliced cooked cured ham is a highly perishable meat product with a low salt content (2.0% in the water phase), a pH value above 6.0 and a water activity higher than 0.95, properties which do not inhibit the growth of spoilage bacteria (Mataragas, Drosinos, & Metaxopoulos, 2003). The number of studies aimed at enhancing the preservation of cooked meats recently have highlighted the role of biocontrol using lactic acid bacteria (LAB) as a protective culture to inhibit the growth of spoilage microorganisms (Bredholt, Nesbakken, & Holck, 2001). Protective cultures are micro-organisms that can suppress the growth of undesirable micro-organisms in a number of ways such as by competition for nutrients, the production of organic acids and hydrogen peroxide, antimicrobial enzymes, bacteriocins, reuterin, etc. (Kotzekidou & Bloukas, 1995).

The LAB is generally considered to be healthful micro-organisms, and show particular potential for selection and implementation as protective cultures. In Western Europe, *Lactobacillus sakei* is generally considered to be beneficial. It plays a crucial role in meat preservation due to its strong ability to compete for growth requirements or lactic acid production or the synthesis of compounds which are inhibitory to pathogenic or spoilage bacteria (Champomier-Vergès, Chaillou, Cornet, & Zagorec, 2001). In 1995, Holzapfel, Geisen, & Schillinger isolated *Lactobaciiius sakei* from meat and used the isolate to inoculate sliced bologna type sausages and found that it successful inhibit the growth of *Listeria monocytogenes* which commonly occurs in this type of sausages. Several investigations into the improvement of the shelf-life of cooked meat products

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have shown that *L. sakei* is efficient as a protective culture (Aymerich, Garriga, Costa, Monfort, & Hugas, 2002). Vermeiren, Devlieghere, and Debevere (2006) reported that *L. sakei* 10A, isolated from cooked turkey fillet, inhibited the growth of representative spoilage bacteria *Leuconostoc mesenteroides* (LM4) and *Brochothrix thermosphacta* (BT1) in co-culture studies on vacuum packaged cooked ham stored at 7 °C.

To fulfill industrial requirements, a protective culture should be easy to culture, easy to apply to the product and give reliable and reproducible results (Bredholt et al., 2001). It is crucial that the effects of the protective culture on the inhibition of growth of the spoilage organisms in the meat matrix are characterized in detail and that the dynamics of the bacteria population during storage of meat products is completely elucidated. However, research into these protective effects in meat and meat products often uses conventional microbiological testing methods which are labor intensive and time consuming, and often require a number of different culture media for final isolation and confirmatory tests which, frequently lead to confusing results. The development of the polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) technique has facilitated the study of microbial populations and their diversity as well as the identification of bacterial strains (Ampe & Miambi, 2000; Muyzer, De Wall, & Uitterlinden, 1993). In the last decade, PCR–DGGE has been successfully applied to investigate the microbiology of fermented foods (Ampe, Ben Omar, Moizan, Wacher, & Guyot, 1999; Cocolin, Manzano, Aggio, Cantoni, & Comi, 2001; Temmerman, Masco, Vanhoutte, Huys, & Swings, 2003) and has also been used to characterize the dominant spoilage bacteria in beef or chilled pork during refrigerated storage (Ercolini, Russo, Torrieri, Masi, & Villani, 2006; Fontana, Cocconcelli, & Vignolo, 2006; Li, Zhou, Xu, Li, & Zhu, 2006; Russo, Ercolini, Mauriello, & Villani, 2006). Nevertheless, to our knowledge, few studies have investigated the protective effects of LAB against spoilage bacteria in cooked meat products using PCR–DGGE technology. Therefore, the objective of this study was to evaluate *L. sakei* B-2 as protective culture for its usefulness in prolonging the shelf-life of vacuumed packaged sliced cooked ham stored at 4 °C. The purpose of this study was to establish whether there was an inhibitory interaction between protective cultures and target strains, and to characterize the dynamics of these microorganisms during storage of meat products under refrigerate conditions.

2. Materials and methods

2.1. Preparation of the protective culture

The protective culture used was a commercial culture Bactoferm™ B-2 of *Lactobacillus sake* obtained from Chr. Hansen (Copenhagen, Denmark). The lyophilized culture was reconstituted according to the manufacture's instructions: 25 g of the culture was dispersed in 500 ml sterile deionized water and was held at 20 °C for 30 min, and was then refrigerate at 4 °C. The sample of sliced cooked ham was treated with the culture at a concentration of 0.25 ml per 100 g meat product to which is equivalent to approximately 10^6–10^7 CFU g^{-1}.

2.2. Production and sampling procedures

Sliced cooked pork hams were prepared in a local meat factory according to conventional techniques without the addition of any preservatives. The ham was made with pork meat, sodium chloride, pentasodium tripolyphosphate, sodium ascorbate, sodium glutamate, sucrose, flavoring additives, soya isolate protein, potato starch, and water. The raw materials were mixed, cured for 16 h, and then packed into an artificial casing and cooked until the core temperature reached 72 °C, the product was then smoked for 2 h. After cooling with cold water for half hour to a core temperature of about 20 °C, the products were maintained at 4 °C overnight. The hams were then aseptically sliced into 0.5 cm slices, and a suspension of the LAB culture was evenly sprayed onto both sides of each sliced of ham, ensuring that the total amount of suspension in the package was appropriate for the weight of ham in the sample. After vacuum packaging with polyamide (PA)/polyethylene (PE) membrane (oxygen permeability $<24\ cm^3\ m^{-2}\ day^{-1}$ at 20 °C, 120 μm thickness with PA/PE ratio 20/100), 100 packages (100 g sliced hams, per package) were stored at 4 °C together with a further 100 packages (100 g sliced hams, per package) which had not been treated with the protective culture. The treated and non-treated ham were sampled at 1, 3, 7, 15, 25 and 35 days for microbiological, pH analysis and sensory evaluation, and both the control and experimental ham was sampled on days 1, 7, 15, 25, 35 and 45 for molecular analysis.

2.3. pH measurements

Two grams portion of sample (two replicates) was homogenized in 18 ml distilled water (pH 7.00), and the pH measured using a Hanna HI9025c Microprocessor pH meter (Romania). Three independent values were obtained for each sample, and means and standard deviations were calculated.

2.4. Microbiological analysis

Using aseptic techniques, 25 g of each sample (two replicates) were homogenized in 225 ml of sterile peptone saline (1 g of peptone and 9 g of NaCl per liter water). After shaking at 230 rpm for 10 min with a stomacher, this suspension was serially diluted in triplicate (1:10) in peptone saline, and 1 ml dilutions were inoculated onto Plate Count Agar (PCA agar, LuQiao Company, BeiJin, China) to obtain the total aerobic count, and onto de Man Rogosa Sharpe agar (MRS, Oxoid, Britain) to determination of lactic acid bacteria. Plates were then incubated for 48 h

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