Screening for cultures usable for biopreservation of meat products

by

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Background

Biopreservation is a way to enhance the safety of food by using a selected microflora and its antimicrobial metabolites. In vacuum packed meat products lactic acid bacteria (LAB) become the dominant microflora during storage. Sometimes the dominating LAB cause spoilage of the product, however, in other cases the product remains fresh throughout storage. In the last decades the preservative potential of bacteriocin producing LAB has gained enormous interest (Gorris & Bennik, 1994; Holzapfel *et al.* 1995; Jack *et al.* 1995; Schillinger *et al.* 1996; Stiles & Hastings, 1991). Much of this work has focused on the antimicrobial effect in laboratory media. However, the ability of bacteriocin producing LAB to inhibit the growth of pathogenic (Schillinger *et al.* 1991) and spoilage bacteria (Leisner *et al.* 1996) in meat has been studied.

If the use of bacteriocin producing LAB cultures is going to be a success in meat products, the cultures must be able to grow at low temperatures, produce active bacteriocins inhibitory to pathogenic and spoilage bacteria and the growth must not cause undesirable organoleptic changes in the products.

Objectives

The aim of the present study was to isolate cultures which can be used to improve the safety of sliced cooked meat products stored at low temperatures without changing the organoleptic properties of the final products.

Materials and methods

Screening

Commercially produced sliced and vacuum packed meat products such as ham, salami and smoked bacon were screened for bacteria with antimicrobial activity. Only products with acceptable sensoric properties at the end of the declared shelf life were analysed. 10 fold dilutions of each product were spread on Brain Heart Infusion (BHI), Man de Rogosa & Sharp (MRS) and All Purpose with Tween (APT) agars and incubated at 10°C and 30°C. Agar plates with approximately 50 colonies were overlaid with soft agar seeded with *Listeria monocytogenes, Leuconostoc mesenteroides, Brochotrix thermosphacta, Escherichia coli* or *Salmonella typhimurium*. Only colonies with inhibitory activity against one of the five indicator strains were isolated for further characterisation and identification. The production of bacteriocin was indicated by the agar spot assay (Spelhaug & Harlander, 1989) by adding 5 µl of proteinase K and pronase E next to the producer organism before overlaying with the inoculated soft agar.

Flavour compounds

The bacteriocin producing cultures were pelleted by centrifugation and re-suspended in physiological saline with 0.1% peptone to OD_{620}= 0.3-0.5. Ten ml of this suspension was added to 100 g of a meat model system containing 3 % NaCl. The inoculated samples were stored at 7°C for 10 and 20 days. The samples were cut into small pieces and extraction of volatile compounds and chromatographic separation were carried out with head space gas chromatography as described by Hinrichsen og Pedersen, 1995. The results were examined by principal component analysis (PCA) in the Unscrambler (CAMO A/S, 1994).

Bacteriocin production

A meat model system containing 3% NaCl was inoculated with 7 log cfu/g of the bacteriocin producing LAB (n=9), vacuum packed and stored at 10°C for 6, 9 and 13 days. From each sample 50 g was stomached for 1 minute in 5-10 ml 0.02 M HCl. One ml of the suspension was centrifuged and the antilisterial activity of the cell free supernatant was measured in a radial well diffusion assay with BHI agar seeded with *L. monocytogenes* at a level of 5 log cfu/ml and incubation at 20°C.

Antilisterial activity of a lactic acid bacteria

A meat model system containing 2% NaCl was inoculated with a lactic acid bacterium at a level of 7 log cfu/g and a cocktail of five strains of *L. monocytogenes* at a level of 4 log cfu/g. The five strains of *L. monocytogenes* were isolated from meat products and mixed 1:1:1:1:1. The samples were vacuum packed and stored at 10°C for 4 weeks with sampling after 1 day, 1 week, 2, 3 and 4 weeks. Total count was measured on BHI agar (20°C/5 days) and the number of *L. monocytogens* was measured on Oxford agar (37°C/2 days).

Results and discussion

Screening

As the production of bacteriocin depends on the growth conditions (Lewus & Montville, 1992; Yang & Ray, 1994) this screening was carried out on three different media and at two different temperatures. Screening at 10°C was chosen because suitable cultures has to produce bacteriocin at low temperatures in order to be usable for biopreservation of cold stored meat products.

During the screening 48 different products were analysed for the presence of bacteria producing antimicrobial metabolites. Bacteria with antimicrobial activity were isolated from 34 different products and bacteriocin producing strains were detected in 22 of these products.

Furthermore, they seem to compete well in the products as they were present in high numbers (5–6 log cfu/g) at the end of shelf life. 151 colonies showed inhibition of one or more of the five indicator organisms (Table 1). These colonies all belonged to LAB characterised as being catalase negative and gram positive. The antimicrobial activity of 43 strains was sensitive to proteolytic enzymes indicating that the inhibition was due to bacteriocins. In the further work only 14 different cultures were included.

Most of the antimicrobial strains were isolated from different types of ham whereas only a few were found in salami and bacon. *L. monocytogenes* was the indicator strain most frequently inhibited (Table 1).

Table 1. Number of cultures with antimicrobial activity found at the screening against five different indicator strains and the number of bacteriocin producing strains isolated.

Indicator strain inhibited	*Strains isolated at*		*Bacteriocin producing strains*
	10°C	*30°C*	
Listeria monocytogenes	*23*	*57*	*28*
Leuconostoc mesenteroides	7	11	12
Brochotrix thermosphacta	5	13	3
Salmonella typhimurium	2	19	0
Escherichia coli	0	16	0

Flavour compounds

Time of storage had no influence on volatile compounds produced by the different cultures as PCA showed no difference in volatiles. The scores plot showed that the cultures could be placed in two groups. Eleven cultures were placed in one group characterised by no production of volatiles compared to non-inoculated samples. Three cultures were placed in another group. The loading plots showed that these three cultures were characterised by the production of methyl branched aldehydes and alcohols (data not shown).

Bacteriocin production

The nine cultures testet in this experiment where chosen because they were able to produce bacteriocins inhibitory to *L. monocytogenes* when grown on BHI agar at 10°C. The results showed that only five cultures produced extractable bacteriocins in the meat model.

Antilisterial activity of a lactic acid bacterium

One of the cultures, which did produce an extractable bacteriocin in the meat model system and which did not produce any undesired aroma, was chosen for investigation of the antilisterial activity in a meat model system. The results showed that the *L. monocytogenes* count was reduced from 4 log cfu/g to below 1 log cfu/g after two weeks storage at 10°C. After 3 and 4 weeks storage a few survivors of *L. monocytogenes* were detected in one or two of the three samples measured (Figure 1).

Conclusion

This study shows that LAB producing bacteriocins are very common in vacuum packed meat products. The analysis of the volatile compounds showed that most of the cultures are suitable for biopreservation as they do not produce any aroma. Extractable bacteriocins were produced in meat model systems by five strains and one of these strains was able to reduce the number of *L. monocytogenes* in vacuum packed meat.

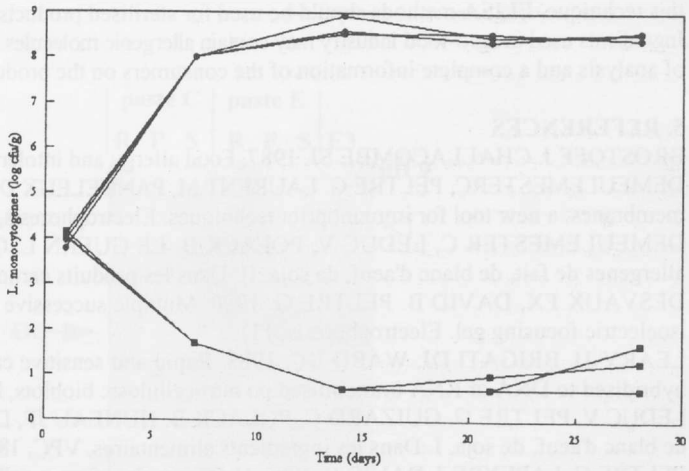

Figure 1. Antilisterial activity of a lactic acid bacteria in a meat model system incubated at 10 °C. (■) Listeria monocytogenes + lactic acid bacteria; (●) Listeria monocytogenes.

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