PROTECTIVE CULTURES INHIBIT GROWTH OF *LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI* O157:H7 IN COOKED, SLICED, VACUUM- AND GAS-PACKAGED MEAT.

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Background

Contamination of cooked meat products with *Listeria monocytogenes* poses a constant threat to the meat industry. In 1992, six cases (one fatal) of listeriosis due to *Listeria monocytogenes* serotype 1/2 following consumption of cooked sliced meat products were reported in Norway (Nesbakken, 1995) and 297 cases (63 fatal) in France were caused by *L. monocytogenes* serotype 4b from pork tongue in aspic (Goulet et al., 1993). Vacuum or gas-packed cooked sliced meat products in Norway have pH values of approx. 6.2, salt contents from 2.5 - 3% (in water phase) and water activities of 0.97 or higher. Thus these products have little or no inherent stability against the growth of *L. monocytogenes*. Lactic acid bacteria (LAB) may be used as a protective microflora to inhibit the growth of *L. monocytogenes* and other undesired organisms, and thus extend storage life and increase safety of food products (Bredholt et al., 1999). Protective cultures should alter the sensory properties of the product as little as possible. *E. coli* O157:H7 is an emerging food pathogen capable of growth at ≤ 10 °C (Buchanan and Doyle, 1997). Cross contamination of vacuum- or gas-packaged meat with either of these bacteria could therefore have serious consequences in meat products.

Objective

The aim of this study was to investigate the use of indigenous lactic acid bacteria as protective cultures in cooked meat products to inhibit growth of food pathogens.

Methods

Wild - type and rifampicin - resistant mutants (*rif* mutants) of three different strains of *L. monocytogenes* (strain 2230/92 serotype 1, strain 169, serotype 4b, strain 187, serotype 4b) were grown in Brain Heart Infusion broth. Plate counts of *rif* mutants were performed on blood agar with 100 µg/ml rifampicin. *E. coli* O157:H7 NCTC 1200, a non-toxigenic and nalidixic acid streptomycin (NAS) resistant strain was grown TSY at 37°C. Plate counts of NAS resistant - mutants were performed on Blood agar with 50 µg/ml nalidixic acid and 1000 µg/ml streptomycin sulphate. *Rif* - resistant mutants of three different strains of *Y. enterocolitica* serotype O:3 were grown in TSY at 30°C. Plate counts of *rif* mutants were performed on blood agar with 100 µg/ml rifampicin. Growth rates of all *rif* - resistant mutants were comparable with those of the wild type strains. Colony counts of LAB were made on MRS agar. *L. sake* strains were identified by hybridisation to species-specific oligonucleotide probes. Freshly made cooked, sliced, vacuum- or gas-packaged ham and servelat sausage (a Norwegian non - fermented cooked meat sausage) from 9 meat plants in Norway were inoculated by injecting 0.1 ml of a cocktail of the three *rif* mutants of *L. monocytogenes*, giving approximately 10^3 cfu/g, through gas probe sealing tape and stored at 8°C for four weeks. Growth of *L. monocytogenes*, the total number of aerobic and lactic acid bacteria and changes in pH were followed throughout the storage period. Samples were analysed after 0, 3, 14 and 28 days. Bacterial counts were determined using two parallel packages for each sampling point. When evaluating the efficiency of protective LAB cultures, cooked ham was inoculated with 10^4 cfu/g of the five LAB test strains (one strain per package) and 10^3 cfu/g of a *L. monocytogenes* cocktail or 10^2 -10^3 cfu/g of the *E. coli* O157:H7 NAS mutant strain. Growth and pH changes were measured after 0, 3, 7, 14 and 28 days at 8 °C or 10°C, respectively. Sensory analyses were carried out in compliance with ISO 6564-1985. Samples were evaluated according to the intensity of odour, colour, flavour and texture.

Results and discussion

When *L. monocytogenes* was added to cooked ham, the pathogen failed to grow on hams from six of nine meat plants. Five different *L. sake* strains were selected for evaluation as potential protective cultures from these packages according to their ability to grow at 3°C, at pH 6.2 and pH 5.8 and with 3% NaCl added to the medium. Cooked, sliced, vacuum - packaged ham was inoculated with *L. monocytogenes*, and 10^4 cfu/g of each of the five selected LAB strains and stored for 28 days at 8°C. After three days, the LAB had increased in number from 10^4 cfu/g to 10^7 – 10^8 cfu/g (Fig 1A). In the control packages with no added LAB, *L. monocytogenes* grew to 10^8 - 10^9 cfu/g within 28 days (Fig. 1B); the numbers of indigenous LAB bacteria were initially below the detection limit (<100 cfu/g), but increased to 10^7 - 10^8 cfu/g after two weeks and to 10^8 - 10^9 cfu/g after four weeks. Addition of 10^4 - 10^5 cfu/g LAB also had an inhibitory effect on the growth of 10^3 cfu/g *E. coli* O157 H:7 in cooked ham stored at 10°C for four weeks (Fig. 1C). After 14 days statistically significant differences ($p<0.05$) were observed between the levels of *E. coli* O157 H:7 in the packages to which LAB were added compared to control packages without LAB. No significant growth of *E. coli* O157:H7 was observed upon prolonged storage after 14 days at 10 °C in the samples with added LAB. In the absence of added LAB, *E. coli* O157 increased in numbers to 10^{5-6} cfu/g. We did not, however, observe any reduction in the numbers of *E. coli* O157:H7 in the presence of the LAB. Taking into account the low infectious dose (2 – 2000 cells) of this pathogen (Buchanan and Doyle, 1997), it is particularly important to eliminate any risk of cross - contamination in cooked meat products, which are consumed without further heat treatment.

Addition of 10^4 - 10^5 cfu/g of the five LAB strains did not inhibit growth of *Y. enterocolitica* on cooked sliced vacuum-packaged ham stored for 28 days at 8°C (not shown). Some results indicate that the ability of *Y. enterocolitica* to compete with other psychrotrophic organisms normally present in foods may be poor (Schieman, 1989). However, our results indicate that 10^4 cfu/g of *Y. enterocolitica* is able to grow well at 8 °C in vacuum-packaged cooked ham and servelat sausage in the presence of 10^{4-5} cfu/g LAB. It is therefore important to avoid cross - contamination with this bacterium.

Sensory analysis was performed on cooked ham inoculated with 10^6 cfu/g of the five test lactic acid cultures and stored at 8°C for 21 days. On comparison with the control packages, all of the inoculated packages were found to be acceptable. Ham inoculated with LAB strains had a slightly more sour taste and/or sour smell than the controls.

Inhibition of undesired or pathogenic microorganisms by LAB may be due to the effect of one, or synergism between, several mechanisms. Competition for nutrients, lowering of pH, production of lactic acid, acetic acid, hydrogen peroxide or other anti-

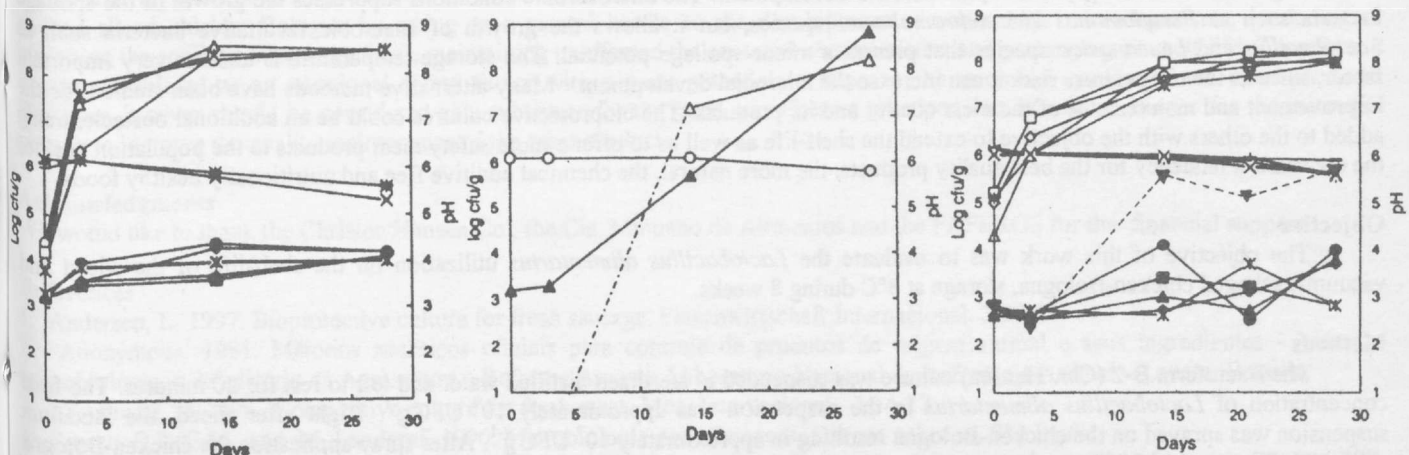

Fig. 1. A: Inhibition of *L. monocytogenes* on cooked, sliced, vacuum-packaged ham at 8 °C after addition of 10^4 cfu/g of five selected LAB strains. B: Growth of *L. monocytogenes* on cooked, sliced, vacuum-packaged ham at 8 °C without added LAB. C: Inhibition of *E. coli* O157:H7 on cooked, sliced, vacuum-packaged ham at 10 °C by addition of 10^4 - 10^5 cfu/g of selected LAB strains. Growth of *E. coli* O157 on cooked, sliced, vacuum-packaged ham at 10 °C without added LAB (----). Filled symbols: *L. monocytogenes* and *E. coli* O157:H7, open symbols: LAB strains, pH profiles are shown.

microbial substances such as bacteriocins, are examples of such mechanisms (Vandenbergh, 1993). The reason for the observed inhibition of *L. monocytogenes* remains, as yet, to be determined. However, the LAB strains used were selected specifically for their fast growth rate in the products at temperatures ≤ 8 °C. Faster growth rates and greater competitiveness for nutrients give the LAB a selective advantage over slower growing competitors. An increase in the concentration of undissociated lactic acid was suggested to be the cause of the reduction in numbers of *L. monocytogenes* in vacuum-packaged ground beef after addition of the protective culture *L. alimentarius* FloraCarn L2 (Juven et al., 1998).

Conclusions

Five *L. sake* strains isolated from commercial cooked meat products inhibited growth of a mixture of three strains of *L. monocytogenes*. These LAB strains should therefore be well adapted to growth in the products and to survival in the production facilities. With their inhibitory effect on *L. monocytogenes* and *E. coli* O157:H7, together with their negligible effect on the sensory quality of the products after storage at abuse temperature, the five strains are well suited for application as protective cultures in cooked vacuum-packaged ham and servelat sausage.

Pertinent literature

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