New bioprotective culture with added value

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Background

Since 1991 Chr. Hansen A/S has marketed bioprotective cultures based on *Lactobacillus sakei* to be applied to vacuum-packed and modified atmosphere packed meat products. The strain primarily functions as a competitive bacteria and does not, with the method explored, show bacteriocin production (Juven, 1998). In application tests in a broad range of meat products (Andersen, 2000, Andersen 1997; Andersen, 1995) the culture has proved to inhibit *Listeria monocytogenes* as well as indigenous lactic acid bacteria (LAB) and spoilage bacteria. Nevertheless, one bacteria strain cannot be expected to be applicable and satisfy the demands in respect to sensory assessments to all meat products. Also, with other applications of starter cultures a culture product range is needed. To complicate the bioprotective concept further there seems to be certain geographical differences in the sensory development in the meat products in which the culture has been tested.

Chr. Hansen A/S strongly believes that cultures for bioprotection will play an important role in multi hurdle systems to ensure food safety in the future. Therefore, we are pleased that we can extend our product range with a new bioprotective stain *Leuconostoc carnosum*. The strain has been isolated and patented by the Danish Meat Research Institute (DMRI Patent, 1997). The advantage of this strain is that in addition to competitive exclusion the strain also produces bacteriocin resulting in a bactericidal effect on *L. monocytogenes*.

Listeria spp. are very resistant to various physical and chemical influences. They are also known to aggregate in biofilms and to be rather resistant to antimicrobial agents and consequently, they may easily cause secondary contamination of meats such as cooked foods during handling and packaging. Outbreaks of listeriose have been linked to both raw materials as well as finished products manufactured from meat, poultry, and fish. Although *L. monocytogenes* is widely spread in the environment serious infections are rare. Nevertheless, the mortality rate is more than 50% for people in the risk groups whereas it is only a few percentages for fit people. In average it is estimated that 25% of the cases associated with listeriose outbreaks are fatal (USDA, 2001b, Kröckel, 2000). USDA estimates that 2500 become ill and 500 die of listeriose each year in the USA (USDA, 2001a). It appears that the epidemiology of listeriose occurring within the US is similar to outbreaks outside the US (USDA, 2001b).

Objectives

The present study was undertaken to investigate the anti-listerial effect of *L. carnosum* in cooked vacuum-packed ham. In parallel *L. sakei* (Bactoferm™ B-2) and un-inoculated samples were explored. Furthermore, both a single strain as well as a cocktail of five strains of *L. monocytogenes* were explored.

Methods

The following bacterial strains were added to relevant codes: *L. carnosum* (Lc) and *L. sakei* (Ls) at the level of approx. 10^7 CFU/g. *L. monocytogenes* (Lm) V80 was applied as a single strain at the level of approx. 10^3 CFU/g as well as in a blend together with Lm 3G2, Lm P-10, Lm P-01, and Lm P-11. The additional strains were handed over in 1996 from the Danish Veterinary and Food Administration for experimental use.

The brine for injection consisted of 77% water, 9% salt, 6.1% nitrite salt (0.6% $NaNO_2$), 5% phosphate, 2.5% glucose, and 0.12% sodium ascorbate. The ham was produced with 16% weight gain and stepwise cooked to a centre temperature of 72°C. The final weight gain was 10%.

A newly produced cooked ham was diced and inoculated with and without Ls, Lc and Lm at 5°C and vacuum-packed (20% CO_2/80% N_2). The packed products were stored at 5 and 10°C, respectively. The products with the Listeria cocktail applied were only stored at 10°C. The products were analysed the day after inoculation and once a week for 29 days. pH was also measured in the samples without Listeria added (Radiometer, PHM92 with an Ingold electrode).

The products with Lc added were detected by pour plating (enumeration) as well as spread plating (visual recognition and microscopic examination) on BHI (Oxoid), aerobically incubated for three days at 20°C. The products with Ls applied were analysed in the same way on MRS (Oxoid), anaerobically incubated for three days at 30°C. The controls were analysed on both media. Listeria was enumerated on Palcam (Merck) with 2.5% egg yolk emulsion (Oxoid) added, incubated microaerophilic for two days at 37°C. Low levels of Listeria were examined semi quantitatively after enrichment (modified method after McClain & Lee, 1988 and Campanini et al, 1993).

Results and discussion

The newly cooked ham was of an acceptable microbiologically quality. The indigenous bacteria grew differently on MRS (Figure 1) and BHI (Figure 2). The most pronounced differences were found at 5°C. The developments were as seen in other tests and consequently, as expected. Ls and Lc dominated the flora in the codes with bioprotective cultures added throughout the 29 days of investigation. The control codes consisted of a mixed flora.

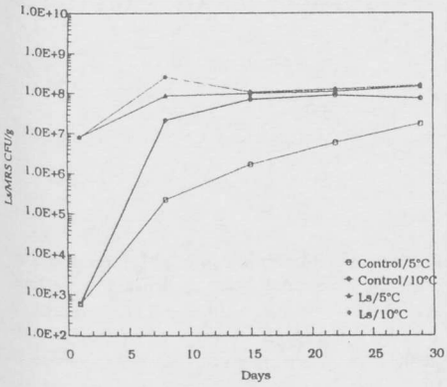

Figure 1. Development of bacterial flora on MRS

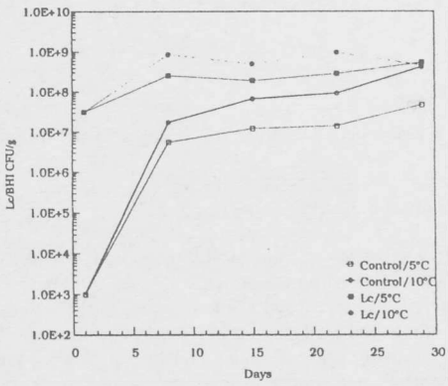

Figure 2. Development of bacterial flora on BHI

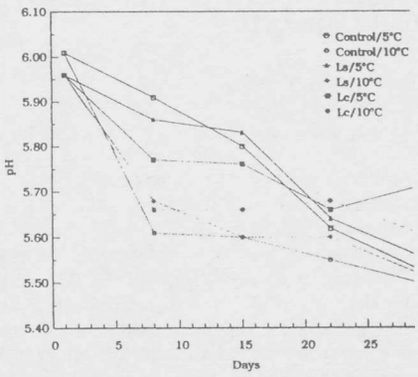

Figure 3. pH development in diced ham during stora

Figure 3 illustrates the pH development in the ham. It is seen that pH is lower when the products are store at 10°C than at 5°C. Furthermore, the control codes followed closely by the meat with Ls added have a lower pH than the codes with Lc applied. Unfortunately, it is difficult to see a clear picture in the pH measurements. The spread in the pH measurements is probably due to the differences in the muscles in the diced ham.

In the products with Lm V80 added without the bioprotective cultures (control codes) Listeria did not grow so fast as seen in other experiments. Nevertheless, Listeria developed and the growth was faster at 10°C than at 5°C, which is normally seen. As displayed in Figure 4 Listeria was inhibited by Ls indicating that a certain degree of safety can be obtained by combining the Ls bioprotective strain with GMP (Good Manufacturing Practise). Furthermore, it is clearly demonstrated that Lc is more efficient to diminish the amount of Listeria compared to Ls.

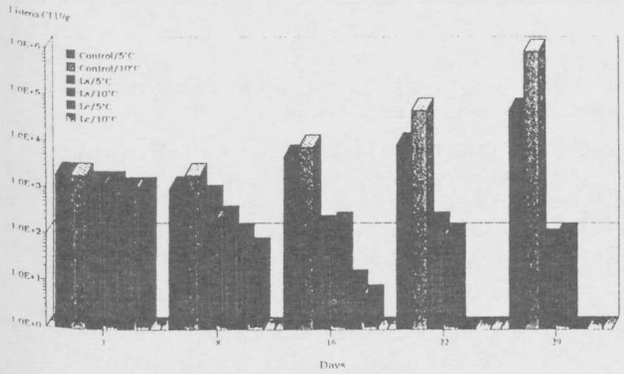

Figure 4. Development of a single strain culture of Listeria during storage

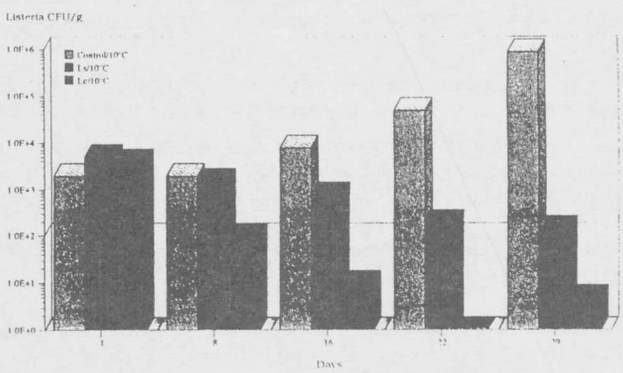

Figure 5. Development of a five strains blend of Listeria during storage

Figure 5 illustrates that it is not specifically Lm V80 that is inhibited by the bioprotective cultures. Also when the cocktail of the five strains of Listeria is applied the same picture is displayed showing an inhibition by Ls whereas Lc lowers the amount of viable Listeria.

Conclusion

Again, the bioprotective concept has proved to inhibit Listeria in meat products efficiently. Chr. Hansen A/S now has two different strains to be applied as bioprotective cultures to meat products. The new strain, *L. carnosum*, inhibits Listeria more efficiently than the present strain, *L. sakei*, as it has been demonstrated in these trials. *L. carnosum* is not only able to inhibit Listeria, the level of Listeria is lowered. The added value of *L. carnosum* is probably due to the bacteriocin produced in combination with competitive exclusion. Further application tests will reveal whether it is possible to apply less Lc initially than the level described in this experiment.

References

References are available on request.