

## Biopreservation of vacuum-packaged sliced Bologna-type sausage by Lactobacilli

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### Summary

Sliced Bologna-type sausage was inoculated with a pool of different serovars of *Listeria monocytogenes* at a level of  $10^3$  /g and, in addition, with  $10^7$  /g of various lactic acid bacteria (LAB) as protective cultures. Three bacteriocinogenic strains of *Lactobacillus sakei*, strains Lb706 (sakacin A +) and strain Lb674 (sakacin P +), and *Lactobacillus curvatus*, strain Lb1071 (curvacin 1071 +), respectively, and a commercial, non-bacteriocinogenic strain, available as 'FloraCarn L-2', were investigated. The bacteriocins produced by the first three strains belong to the "pediocin family" and share an N-terminal -YGNGV- motif. The inoculated sausage slices were vacuum-packaged and stored at 7°C up to 28 days. In the absence of LAB, listerial numbers of  $10^7$  -  $10^8$  /g were reached after 14 - 21 days of storage, while listerial growth was inhibited in the presence of each of the LAB strains. Best inhibition was observed with the bacteriocinogenic LAB strains, all of which were able to reduce the initial inoculum of *L. monocytogenes*. Strongest anti-listerial activity was seen with *L. curvatus* Lb1071. In contrast, the non-bacteriocinogenic commercial strain allowed listerial growth by two decades within 14 - 21 days.

### Introduction

Vacuum-packaged, sliced ready-to-eat meat products with relatively high pH and water activity (pH 6.2,  $a_w$  0.98), where the natural microflora has been inactivated during pasteurization, may represent the main potential field for the application of protective lactic acid bacteria (LAB) in meats. Recontamination during further processing of Bologna-type sausage, cooked ham and pâté may threaten consumer health and product quality. At the same time LAB are most difficult to apply to this products in high numbers without accepting a certain drop in pH. This may however not necessarily constitute a problem, and product quality may actually benefit from the application of an appropriate LAB culture during prolonged storage if compared to uninoculated sausages (1, 2).

When using lactic acid bacteria (LAB) as protective cultures in the biopreservation of cooked meat products, the inoculation level is very important. We found that in vacuum-packaged, sliced Bologna-type sausage stored at 7°C, low initial numbers of *L. sakei* were not sufficiently active to suppress the growth of *Listeria monocytogenes* (L.m.), even if a potent anti-listerial bacteriocin was included in the recipe. Good inhibition of L.m. - but no reduction - was found when  $10^5$ - $10^6$  cfu/g of bacteriocinogenic *Lb. sakei* were applied, while a pool of 20 random LAB isolates from this type of meat product gave no protection under these conditions (1, 5). In comparison, FloraCarn L-2, a bacteriocin-negative commercial culture, was reported to show a satisfying effect at 5°C when inoculated at a level of at least  $10^7$  cfu/g (2).

The aim of this study was to compare the performance against listeria of some bacteriocinogenic LAB strains and of FloraCarn L-2, a commercial protective culture for meats, on vacuum-packaged, sliced Bologna-type sausage at a storage temperature of 7°C, which is the legal maximum temperature for chilled meats.

## Methods

Bologna-type sausage was purchased from a local producer and sliced under semi-sterile conditions. The procedure of Anderson (1995) was followed for inoculation and vacuum-packaging of the slices. Briefly, sliced Bologna-type sausage was inoculated with a pool of four different serovars of *Listeria monocytogenes* (serovars 1/2c, 4b, 1/2a, 1/2b) at a level of  $10^3$  /g and, in addition, with  $10^7$  /g of various LAB as protective cultures. Three bacteriocinogenic strains of *Lactobacillus sakei*, strains Lb706 (sakacin A +) and Lb674 (sakacin P +), and *Lactobacillus curvatus* strain Lb1071 (curvacin 1071 +), respectively, and a commercial, non-bacteriocinogenic culture, FloraCarn L-2, were investigated. The bacteriocins produced by the first three strains belong to the "pediocin family" and share an N-terminal -YGNGV- motif (3, 4). The inoculated sausage slices were vacuum-packaged and stored at 7°C up to 42 days.

## Results

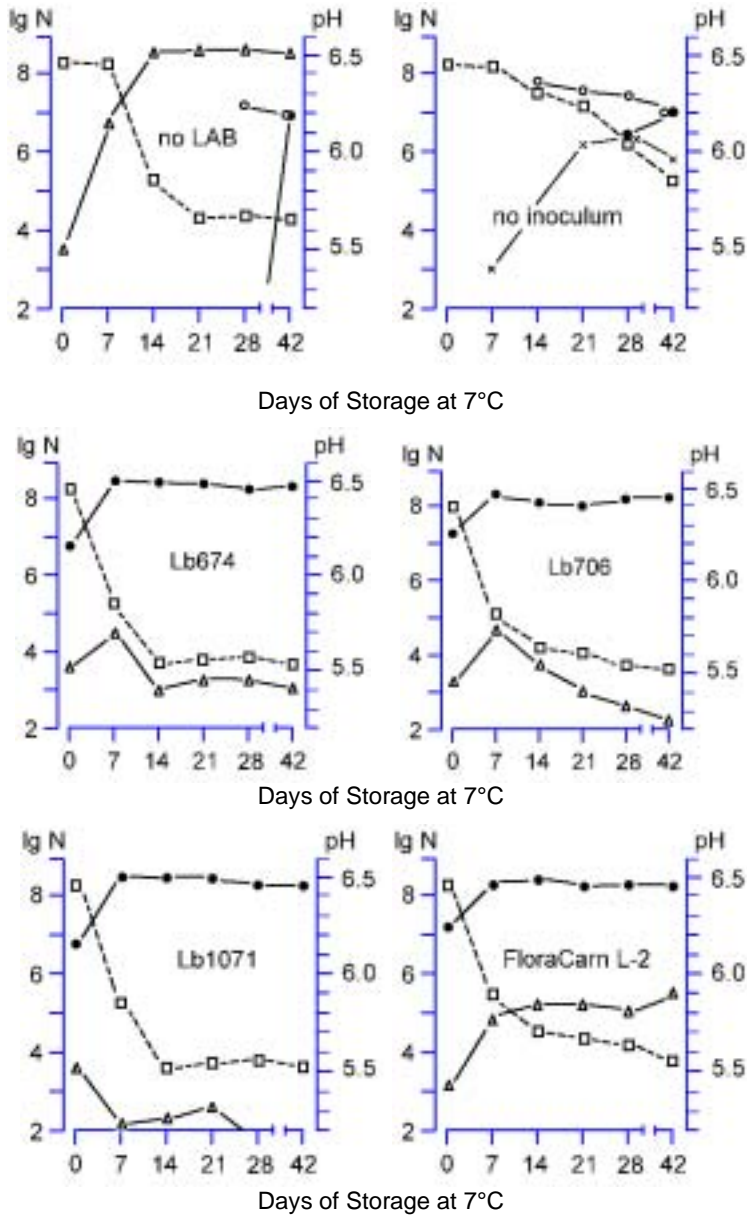
In the absence of LAB, listerial numbers of  $10^7$  -  $10^8$  /g were reached after 14 - 21 days of storage, while listerial growth was inhibited in the presence of each of the LAB strains (Fig. 1). Best inhibition was observed with the bacteriocinogenic LAB strains, all of which were able to reduce the initial inoculum of *L. monocytogenes*. Strongest anti-listerial activity was seen with *L. curvatus* Lb1071. In contrast, the non-bacteriocinogenic commercial strain allowed listerial growth by two decades within 14 - 21 days.

This experiment confirms that 'FloraCarn L-2' at high inoculation levels can prevent excessive growth of *L.m.* in vacuum-packaged, sliced Bologna-type sausage. However, it also shows that bacteriocinogenic strains of *L. sakei* and *L. curvatus* perform much better under such conditions.

Where LAB had been added, the pH decreased from pH 6.5 to values between pH 5.5 - 6.0. For strains Lb674 and Lb1071 pH 5.5 was reached already after 14 days, while Lb706 and FloraCarn L-2 gave a pH drop to pH 5.6 and pH 5.7 at this time, respectively.

For sensoric purpose all trials were run also in the absence of listeria. In spite of the high initial LAB inoculum there were no deviations in smell, taste and appearance after 2 weeks of storage with any of the cultures when compared to the uninoculated control. After 4 weeks, for Lb674, Lb706 and Lb1071 smell was still fresh, appearance good, taste was only slightly more acidic, and the chewing resistance was somewhat less than with the uninoculated control. FloraCarn L-2 and the uninoculated control gave an acidic smell, and an unpleasant taste, which was "rough/pungent, cheesy and old" for the uninoculated control, and "rough/acidic" with an unpleasant aftertaste for FloraCarn L-2. After 6 weeks only samples containing Lb674 and Lb1071 were acceptable, but had a rough/acidic note by then.

*Brochothrix thermosphacta* was less than  $10^6$  -  $10^7$  /g or absent in the presence of LAB, but  $10^7$  -  $10^8$  /g in the absence of LAB after 3 - 4 weeks of storage.



**Fig. 1:** Control of *Listeria monocytogenes* in vacuum-packaged sliced Bologna-type sausage by bacteriocinogenic and non-bacteriocinogenic LAB.

**Legend to Fig. 1:** lg N, log<sub>10</sub> of colony forming units per gram sausage; triangles, *Listeria monocytogenes*; full circles, protective culture (LAB); squares, pH; open circles, *Brochothrix thermosphacta*; crosses, pseudomonads.

Bacteriocinogenic strains: *Lactobacillus sakei* strains Lb674 and Lb706; *Lactobacillus curvatus* strain Lb1071.

Non-bacteriocinogenic strain: FloraCarn L-2.

no LAB: no protective culture added.

no inoculum: no LAB or listeria added.

## Conclusions

At inoculation levels of  $10^7$  LAB/g, the growth of *Listeria monocytogenes* on vacuum-packaged sliced Bologna-type sausage stored at 7°C is completely prevented by the class II-bacteriocin producers *Lactobacillus sakei* strains Lb674 and Lb706, and *Lactobacillus curvatus* Lb1071. The commercial protective culture FloraCarn L-2 efficiently prevents excessive listerial growth but still allows a significant increase of listerial numbers. Since listerial counts even decreased in the presence of the bacteriocinogenic strains, such cultures are clearly preferable over non-bacteriocinogenic ones. All LAB strains used in this study did not deteriorate the taste of the sausages by unacceptable souring.

## References

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