

INFLUENCE OF COMPETITIVE FLORA AND STORAGE CONDITIONS ON GROWTH OF *LISTERIA MONOCYTOGENES*.

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SUMMARY

Inhibitory effect of *Pediococcus pentosaceus* addition on growth of *Listeria monocytogenes* on cured pork loin and pork back fat were investigated. Reduction in development of both Gram negative spoilers, *Brochothrix thermosphacta* and especially *Listeria monocytogenes* was observed at incubation temperatures of 5 °C as well as 10 °C. Incubation in vacuum or CO₂ enriched atmosphere greatly enhanced the effect. At these conditions an initial level of added *Listeria monocytogenes* at 10³ cfu/g rapidly decreased to less than 1 cfu/g within a week.

INTRODUCTION

Listeria monocytogenes has attracted increasing attention due to the fact, that this foodborne pathogen is able to survive and grow at low temperatures and reduced oxygen content. Means to inhibit growth of *Listeria* includes use of organic acids or nitrite addition. Use of a competitive microflora has been evaluated for potential inhibitory effect against *Listeria*. Commercial starter cultures for meat fermentation containing *Pediococci* might have an effect either by generally lowering pH or by production of specific compounds including organic acids, peroxides, diacetyl and bacteriocins. Factors like storage temperature, modified atmosphere and additives are important, because they concert their effect on both pathogens and the competitive microflora. We here report investigations of *Pediococcus pentosaceus* on growth of *Listeria monocytogenes* and the natural occurring flora as well.

MATERIALS AND METHODS

Cured pork loin with nitrite addition; sliced and vacuum packed at the manufacturer in 100 g packages. The packages were randomly divided into four portions and each added 0.3 ml of a suspension of either *Pediococcus pentosaceus* (3.7·10⁴ cfu/ml) or *Listeria monocytogenes* (2·10⁴ cfu/ml) or both (5.7·10⁴ cfu/ml). Controls without inoculation were added sterile water. All packages were vacuum sealed again after inoculation. Storage temperatures were 5 °C and 10 °C.

Pork back fat was bought at a store, and cut using sterile conditions in the laboratory. Each sample was about 14 cm² and weighed app. 10 g. All samples were inoculated with *L. monocytogenes* (10⁴ cfu/cm²). Packages were stored at 5 °C and either exposed to normal atmosphere or CO₂/N₂ atmosphere, using Merck's Anaerocult A system, in anaerobic storage vessels.

Bacteriological examinations:

Pork loin: Once a week two packages from each treatment and both temperatures were examined. 50 g from each package was homogenized with 450 ml peptone/NaCl solution (0.9%) in a Stomacher (medium speed, 2 min's), diluted and spread on agar media. Incubation at 30 °C for 24 hours. Total aerobic counts were made on Plate Count Agar (PCA), lactic acid bacteria on de Man, Rogosa, Sharpe Medium (MRS), *Brochothrix thermosphacta* on Streptomycin Thallous Acetate Actidione Medium (STAA), Gram negative bacteria on Desoxycholate Hydrogensulfide Lactose Medium (DHL), *Listeria* on Listeria Selective Agar Base (LSA) with Listeria Selective Supplement (Oxoid).

Pork back fat: Every third day samples from both aerobic and anaerobic incubation were examined in the same manner as described above for pork loin. The whole fat sample was homogenized in 100 ml peptone/NaCl solution.

RESULTS

Cured pork loin at 5 °C.

Natural flora: The development of a microflora in the vacuum packaged cured pork loin occurs rapidly, the total number reaching 3·10³ cfu/g within the first week and slowly rising to about 10⁵ cfu/g during the 6 weeks storage. The flora is dominated by lactic acid bacteria as well as a considerable number of *B. thermosphacta*, while the Gram negative flora is nearly negligible (Fig. 1.a). Addition of *Listeria monocytogenes* does not alter the general picture (Fig. 1.c). Addition of a starter culture consisting of *Pediococcus pentosaceus* enhances the development of a lactic acid bacteria flora. The total number as well as the number of lactic

acid bacteria reaches 10^5 cfu/g within a week and remains constant throughout the remaining period (Fig. 1.b). Concurrent addition of both starter culture and *L. monocytogenes* results in a slightly higher level of total number of bacteria. The maximum of 10^6 cfu/g is reached within two weeks. *B. thermosphacta* shows a tendency to remain at a fairly low level at about 100 cfu/g for the first four weeks, but ultimately reaches 10^4 cfu/g at the end of the period. Addition of a starter culture seems not to suppress development of *B. thermosphacta* (Fig. 1.d).

Listeria: the number of *Listeria* rapidly decreases in one week. However, the bacteria survives at a low level (less than 1 cfu/g) throughout the period. The addition of starter culture does not seem to influence the number of *L. monocytogenes* present during storage (Fig. 3.a).

Cured pork loin at 10 °C.

Natural flora: The same general picture (Fig. 2.a - 2.d) as at 5 °C is seen here, although the total number reaches slightly higher levels and the flora develops more rapidly.

Listeria: A steadily development of *L. monocytogenes* took place in 3 to 4 weeks finally reaching a level of $8 \cdot 10^4$ to $3.5 \cdot 10^5$ cfu/g, followed by a rapid decrease to 10 cfu/g or less within a week (Fig. 3.b). Addition of *P. pentosaceus* had an effect by lowering the total number of *Listeria* observed, and at an earlier state than was seen without starter addition.

Pork back fat.

The pure pork fat was heavily contaminated from start (10^7 cfu/g), the normal flora consisting of mainly Gram negative bacteria and lactic acid bacteria (Fig. 4.a - 4.b). When inoculated with a high level of *Listeria* (10^3 cfu/g) growth took place during aerobic storage, the number reaching $25 \cdot 10^4$ cfu/g in 3 weeks. Under anaerobic conditions growth is much slower, the level of *Listeria* remains nearly constant at about 10^3 cfu/g throughout the period (Fig. 4.c).

DISCUSSION

In earlier experiments we have shown an inhibitory effect of *Pediococcus pentosaceus* against *Listeria monocytogenes*. The results reported here show inhibition of *Listeria* growth at 10 °C, but not at 5 °C. This is in agreement with DEGNAN et al. (1992), who also failed to show any effect of *P. acidilactici* in vacuum packaged all-beef wieners at 4 °C in 72 days, but a rapid decrease at 25 °C only within a week. The effect was due to bacteriocin production, as a non-producing strain did not cause any decrease in numbers of *Listeria*. In our experiment the inhibition could not be caused by production of acids, as pH remained constant at about 6.3 throughout the period, indicating that bacteriocin production might be the cause for reduction of *Listeria* levels. On the contrary GRAU and VANDERLINDE (1992) have shown, that growth rate of *Listeria monocytogenes* in vacuum packaged ham was only slightly less than the rate of the other flora (mainly lactic acid bacteria) and increasing with storage temperature. At 4.8 °C numbers of *L. monocytogenes* increased 10^4 times in 2 weeks, but growth of *L. monocytogenes* stopped, when the other flora reached a level of about 10^8 /g. The total number of bacteria in our experiment did not exceed 10^7 cfu/g, but in all cases lactic acid bacteria grew faster than *L. monocytogenes*. However, *Listeria* survived at a low level especially at 5 °C, thereby still presenting a potential for renewed growth. This is in agreement with MOTLAGH et al. (1991), who report, that although a *Listeria* strain seems to be sensible to bacteriocins from *P. acidilactici* and others, a certain proportion of resistant *Listeria* survives.

The experiment with pork back fat shows, that also the fat portion of meat might be a substrate for *Listeria*. Storage in modified atmosphere deprived of oxygen lowers the level of *Listeria* in comparison with aerobic storage. But in both cases slow growth is observed even at temperatures of 5 °C. RAZAVILAR and GENIGEORGIS (1992) find no difference between several modified atmospheres including vacuum and 100 % CO₂ after one week, 100 % CO₂ being most effectively in suppressing growth of *Listeria* during prolonged storage. Our results also agree with the findings of MARSHALL et al. (1991), who report of modified atmospheres on *Listeria* growth, the effect being greater with increasing CO₂ content and increasing temperature.

CONCLUSION

Addition of the starter culture *P. pentosaceus* to vacuum packaged ham seems to have an effect by slightly suppressing growth of both *B. thermosphacta* and especially *Listeria* at both 5 °C and 10 °C. The inhibitory effect was not due to a decrease in pH, which remained constant at about 6.3. The nitrite addition might enhance the inhibition. Modified atmosphere deprived of oxygen reduces the growth rate of *L. monocytogenes* on pure pork fat, but slow growth still occurs even at temperatures of 5 °C.

Fig. 1. Cured pork loin. Incubation at 5 °C. a) Control. b) *Pediococcus pentosaceus* addition. c) *Listeria monocytogenes* addition. d) *Listeria monocytogenes* and *Pediococcus pentosaceus* addition.

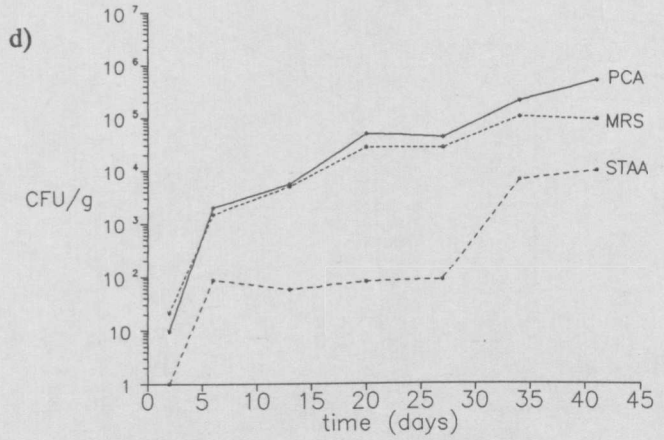
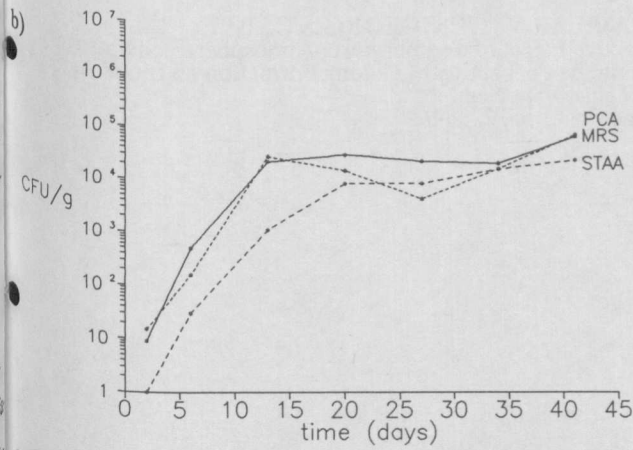
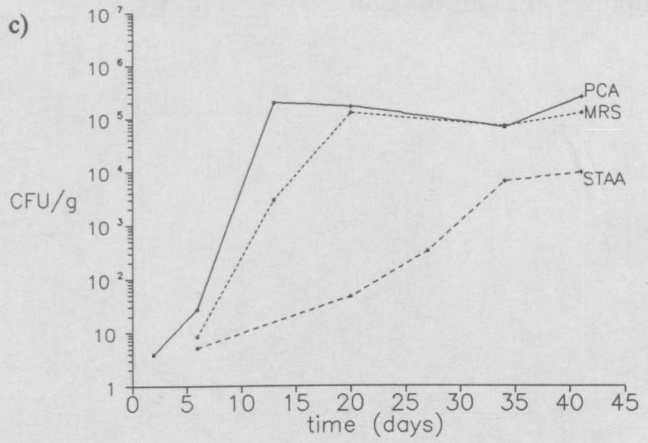
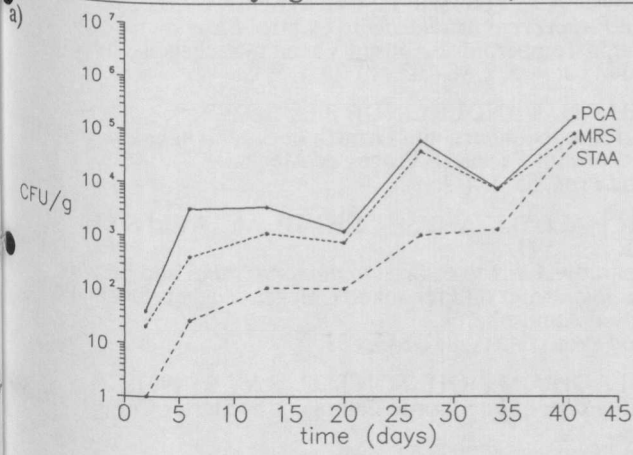


Fig. 2. Cured pork loin. Incubation at 10 °C. a) Control. b) *Pediococcus pentosaceus* addition. c) *Listeria monocytogenes* addition. d) *Listeria monocytogenes* and *Pediococcus pentosaceus* addition.

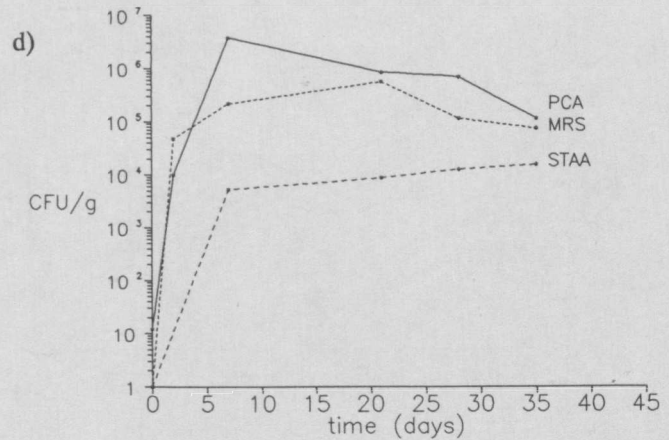
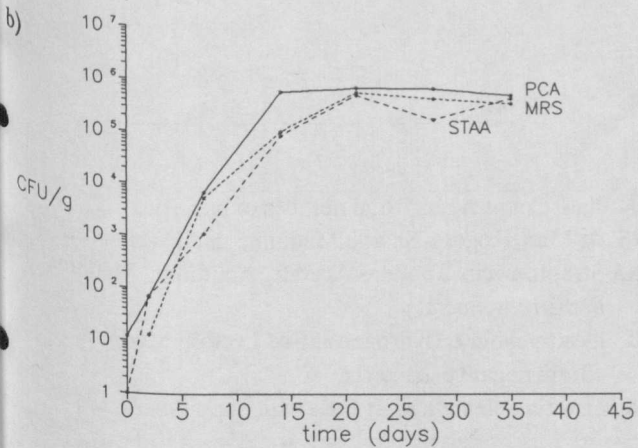
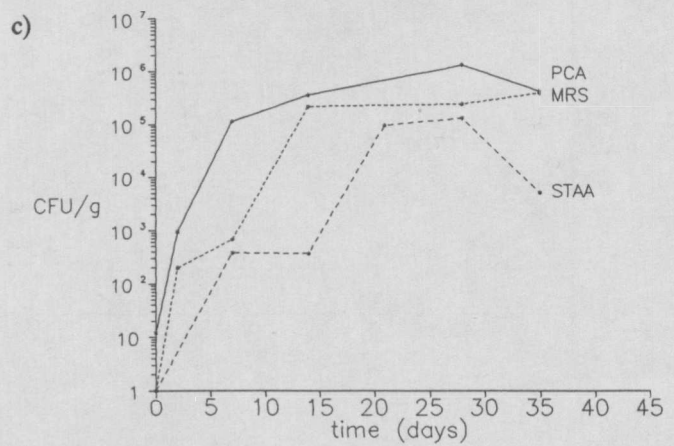
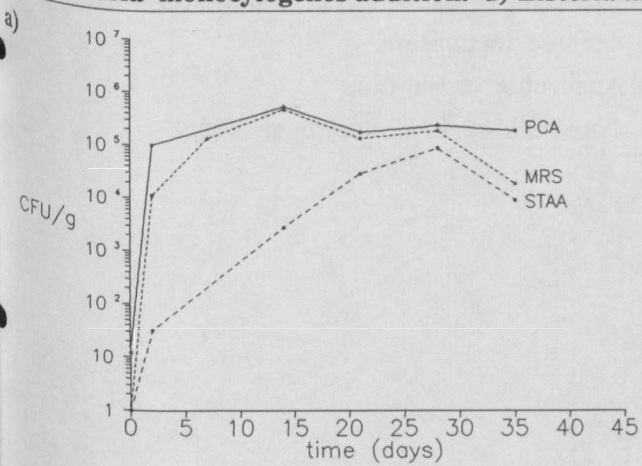
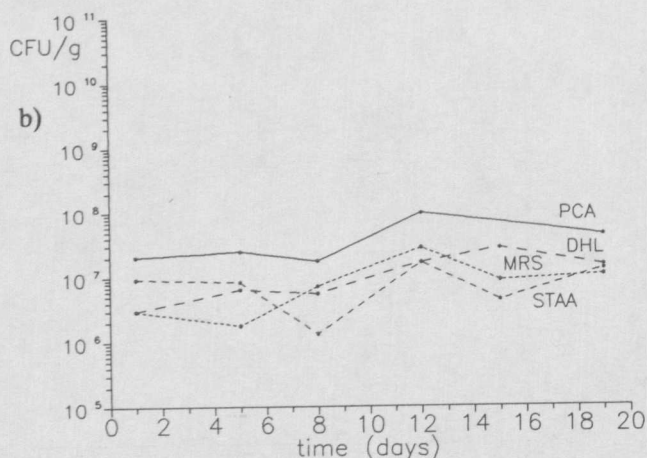
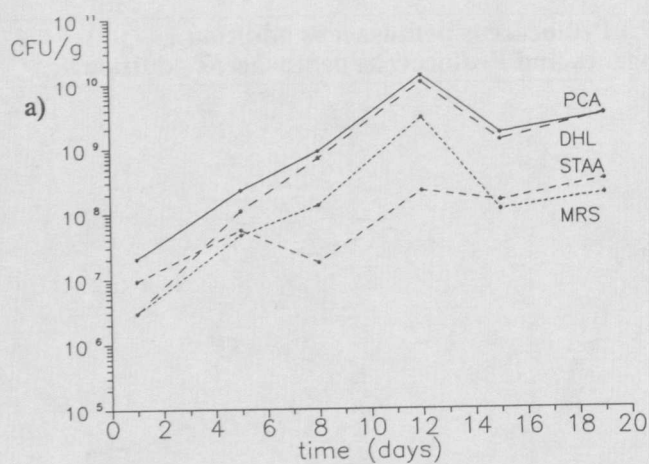
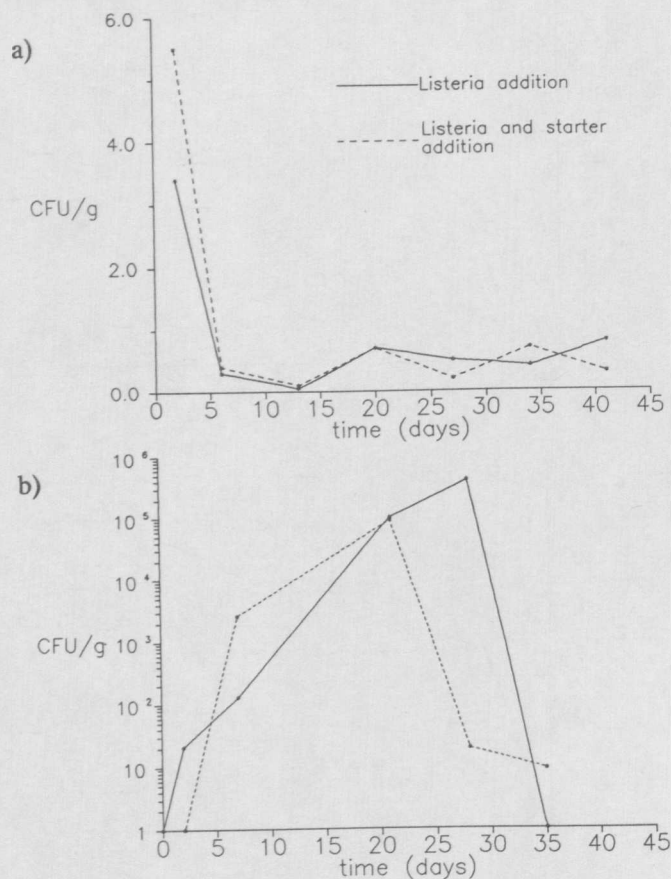


Fig. 3. Cured pork loin. Inoculated with *L. monocytogenes* and/or *P. pentosaceus*. Numbers of *Listeria* (LSA). a) 5 °C. b) 10 °C.



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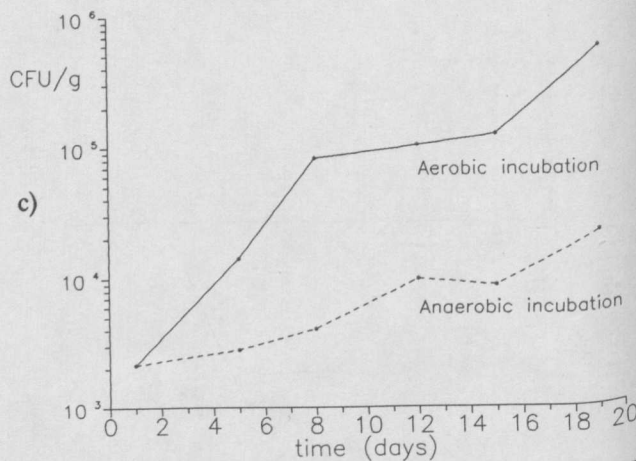
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Fig. 4. Pork back fat. Incubation at 5 °C. Inoculated with *Listeria monocytogenes*. a) Aerobic incubation. b) Anaerobic incubation. c) Numbers of *L. monocytogenes* (LSA).



PCA Plate Count Agar, total number of bacteria.
MRS de Man, Rogosa, Sharpe Medium; lactic acid bacteria
STAA Streptomycin Thallous Acetate Actidione Medium, *B. thermosphacta*.
DHL Desoxycholate Hydrogensulfide Lactose Medium, Gram negative bacteria.
LSA *Listeria* Selective Agar Base with Supplement, *Listeria*.