CONTROL OF Listeria monocytogenes IN MEAT PRODUCTS BY A STRAIN OF Lactobacillus plantarum

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SUMMARY - It was shown that Lactobacillus plantarum MCS isolated from salami produced inhibitor compounds towards Listeria monocytogenes. In a pH-adjusted culture supernatant, the growth of Listeria, monitored by impedometric method at 30°C, was delayed at pH 6.0, while no growth occurred at pH 5.5.
A mutant MCS1 strain showed reduced inhibitor activity. After 6-7 days growth of inoculated Listeria was observed in minced raw pork with added curing agents stored at 18°C and in salami. Therefore the addition of L. plantarum strains prevented Listeria growth during the first few days of meat storage and salami maturation. Listeria counts tended to decrease after 6 and 14 days in all minced meat and salami samples, respectively. At 31 days small differences were observed in the survival of listerias in salami inoculated with L. plantarum MCS or MCS1 (bacteriocin-negative strain).
The inactivation of Listeria was much less effective in meat products than in culture systems, but inoculated Lactobacillus strains prevent L. monocytogenes growth.

INTRODUCTION - Listeria monocytogenes has been shown to occur frequently in raw meat and has also been isolated from cured and fermented products (SCHMIDT et al, 1988, KARCKES and TEUFEL, 1988, FARBER et al, 1988, BARBUTI et al, 1989, FARBER et al, 1989, JOHNSON et al, 1990). A potential means of preserving fermented meats from this pathogen is the use of bacteriocin-producing lactic-acid bacteria as starter cultures.
Inhibition of L. monocytogenes by Pediococcus spp.and some Lactobacillus strains was observed not only in culture media, but also in meat and meat products (HOOVER et al, 1989, SCHILLINGER and LUCKE, 1989, BERRY et al, 1990, SCHILLINGER and LUCKE, 1990, SCHILLINGER et al, 1991, BERRY et al, 1991). Strains of Lactobacillus spp. isolated from dry Italian salami were found to have anti-listeria activity in culture medium (CAMPANINI et al, 1991).

The aim of this study was to assess the bactericidal activity of a L. plantarum strain against L.monocytogenes in a culture medium, in minced meat and in salami during maturation.

MATERIALS AND METHODS

Microrganisms

- L. plantarum MCS isolated from Italian salami and previously selected for anti-listeria activity, was grown in APT medium at 30°C for 24 h. To produce bacteriocin-negative variant (MCS1 strain) the MCS strain was cultured several times in MRS broth with acriflavine (10 micrograms /ml) at 30°C for 24 h.
- L. monocytogenes strains 38 and 150 isolated from salami were grown in Brain heart infusion broth at 30°C for 20 h.

Antagonistic activity in culture media

- Impedometric method: The growth of L. monocytogenes 38 was evaluated by capacitance measurements using a Bactometer Instrument (Bactomatic). The growth of listerias (10^3 - 10^4 cells/ml) was monitored at 30°C in APT broth as control and in filtered (0.2 micron) pH adjusted (6.0 and 5.5) supernatants from 24 h-cultures of Lactobacillus MCS and MCS1 strains. To eliminate the effect of lactic-acid, the APT broth controls were acidified to pH 4.4 with lactic acid, then adjusted to 5.5 and 6.0 with NaOH solution. The growth of Listeria was also monitored in supernatant of MCS strain at pH 6.0 with added protease (1 mg/ml).
The anti-listeria activity was verified at different incubation times in the same culture media by plate count on Trypticase soy agar and Palcam.
- Agar diffusion assay: In the agar spot test the Lactobacillus strains were dotted onto APT agar and after 24 h-anaerobic incubation at 30°C, the agar plates were then coated with 8 ml of soft APT agar (0.7 %) inoculated with 0.2 ml of an overnight culture of L. monocytogenes strains. After 24 h-incubation at 30°C the antagonistic activity was evident from the appearance of a clear inhibition area around the spot colony.

Inoculation experiments

- Minced meat: it was prepared using raw pork mixed with NaCl (3 %), sodium nitrite (150 mg/kg) and sucrose (0.3%). Portions of 30 g each were inoculated with MCS strain (10^6 - 10^7 cells/g) and/or L. monocytogenes 38 (10^3-10^4 cells/g) and placed into sterile plastic bags; after vacuum heat sealing the bags were stored for up to 14 days at 18°C.
- Salami: fresh salami mix containing 2.5 % NaCl salt, 250 mg/kg potassium nitrate and 0.3 % sucrose was inoculated with 24-h cultures of Lactobacillus MCS and MCS1 strains (about 10^7 cells/g) and/or two strains of L.monocytogenes (10^3-10^4 cells/g). After filling into cellulose casings of 50 mm diameter, salami were sprayed with a suspension of Penicillium nalgiovense. The salami were allowed to mature at 18°C for 2 days, at 17-15°C for 3 weeks and at 14°C for 1 week. During maturing pH and aw were measured.

Microbiological determinations

Samples of minced meat and portions of salami (about 50 g each) were mixed with a sterile solution (0.85 % NaCl and 0.1% peptone) and homogenized in a stomacher. L. monocytogenes numbers were determined on Palcam agar (2 days at 37°C) and confirmed by motility and emolysis tests. The lactobacilli were enumerated on Rogosa SRL agar (3 days at 30°C).

RESULTS AND DISCUSSION - The impedometric method showed that the growth of L. monocytogenes 38 was inhibited in a culture supernatant of L. plantarum MCS (Fig.1). At pH 6, a delay of 32 h in detection time was observed; on the contrary, at pH 5.5 no growth occurred in 96 h (flat impedometric curve). The anti-listeria activity of the culture supernatant was lost when this was treated with pronase, proving that a protein or a proteinaceaus compound is probably responsable for the antagonistic effect.

An agar spot test was used to screen, after acriflavine treatment, mutant strains of L. plantarum MCS by their reduced inhibitor activity. Only one strain (MCS1) among the selected ones, showed little residual antilisteria activity by impedometric method (Fig.2). The delay in detection time in pH 6-adjusted supernatant dropped from 32 to about 5 h.

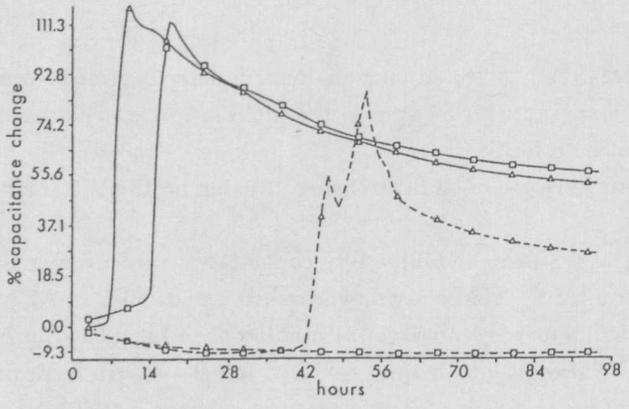

Fig. 1 - Growth of L. monocytogenes 38 at 30°C in APT control (—) and in filtered supernatants of a culture of Lactobacillus MCS (----).
△ adjusted to pH 6.0
□ adjusted to pH 5.5

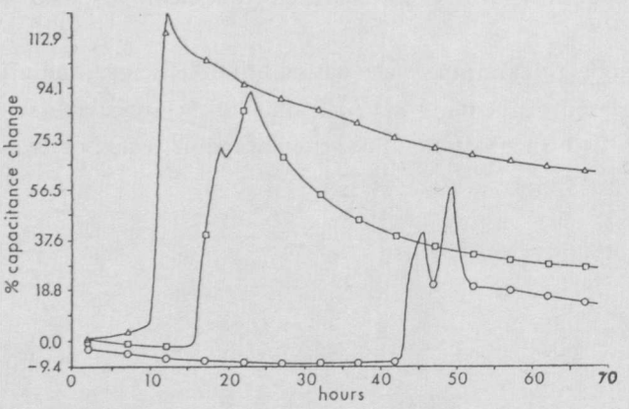

Fig. 2 - Growth of L. monocytogenes 38 at 30°C in media adjusted to pH 6.0.
△ APT broth control
□ filtered supernatant of a culture of Lactobacillus MCS1
○ filtered supertantant of a culture of Lactobacillus MCS.

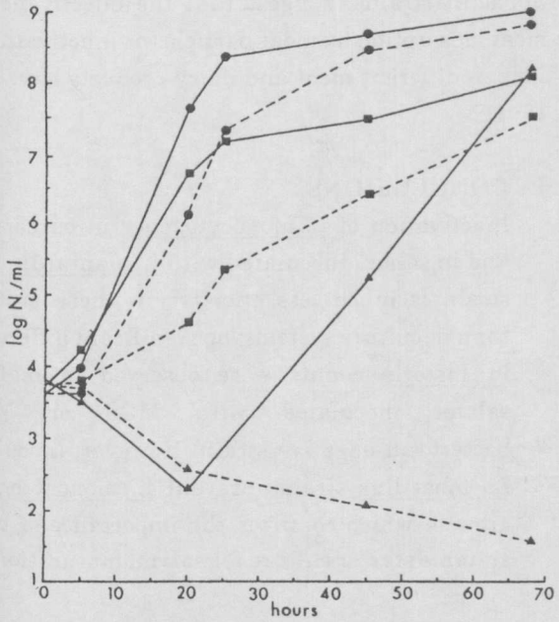

Fig. 3 - Growth and survival of L. monocytogenes 38 at 30°C in media adjusted to pH 6.0 (—) and 5.5 (----)
● APT broth control
■ filtered supernatants of a culture of Lactobacillus MCS1
▲ filtered supernatants of a culture of Lactobacillus MCS

The antagonistic effect of MCS strain verified by plate counts of surviving listerias is shown in Fig.3. L. monocytogenes grew faster in MCS1 supernatant at pH 6.0 and more slowly at pH 5.5; slow delayed growth was observed in MCS supernatant at pH 6.0, while at pH 5.5 Listeria was inactivated. At this pH value a bactericidal effect previously observed for a similar strain (CAMPANINI et al, 1991) was confirmed, proving a synergic antagonistic action .

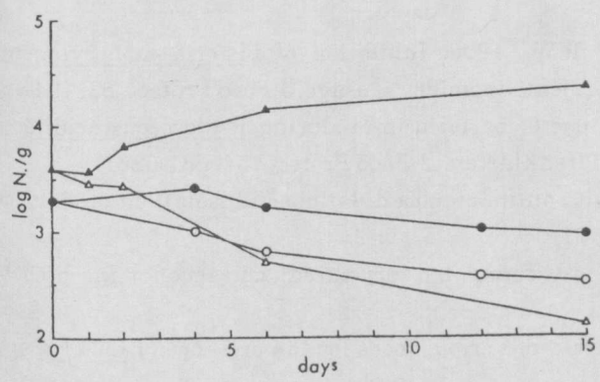

Fig. 4 - Behaviour of L. monocytogenes in minced raw pork at 18°C.
No added lactobacilli, pH 5.8 (▲) and 5.6 (●)
In presence of L. plantarum MCS, pH 5.8 (△) and 5.6 (○).

The results obtained in minced meat (pH 5.8 and 5.6) are shown in Fig.4. In samples inoculated with MCS strain L. monocytogenes counts declined by 0.8 and 1.3 logarithmic cycles in 15 days at 18°C in meat at initial pH 5.6 and 5.8, respectively. On the contrary, in samples inoculated only with Listeria, the counts declined by a 0.3 logarithmic cycle in meat at pH 5.6, while L. monocytogenes grew in meat at pH 5.8. Recently SCHILLINGER et al (1991) observed a rapid growth of listerias at 15°C in comminuted cured raw pork at high pH (6.3), while no growth occurred at pH 5.7, independently of inoculum level.

During maturing, in salami inoculated only with Listeria after 7 days the lattobacilli naturally present increased from about 10^3 to more than $5*10^7$, but the pH value remained almost constant at 5.7-5.8 and a growth of listerias (an increase of about ten fold) was observed (Fig.5). However, in the presence of L. plantarum MCS and MCS1 strains, pH decreased to 5.3 after 7 days and the viable number of L. monocytogenes remained more or less constant. After 14 days, pH values were unchanged but Listeria counts tended to decrease in all samples; at 31 days in salami inoculated only with Listeria (pH 5.8) the survival counts came back to the initial level, while in the samples inoculated with lactobacilli (pH 5.5), a reduction of about 0.7 and 0.6 log cycles was observed with MCS and MCS1, respectively. The small differences in the

survival of listerias, in salami inoculated with two different lattobacilli strains, suggest that the effectiveness of bacteriocin may be reduced by a limited diffusion of the protein in meat, adsorption by meat particles or inactivation by proteolytic enzymes produced during maturation. Similar observations in different meat and dairy products have been reported by other authors (PUCCI et al, 1988, SCHILLINGER et al, 1991).

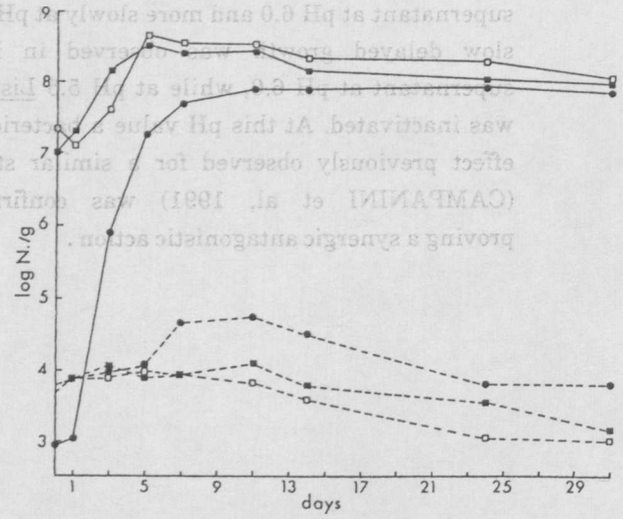

Fig. 5 - Behaviour of L. monocytogenes (-----) and lattobacilli (——) in salami during maturation.
● no added lattobacilli
■ in presence of L. plantarum MCS1
□ in presence of L. plantarum MCS

CONCLUSIONS

Inactivation of L. monocytogenes in cured meat and in salami inoculated with L. plantarum MCS strain is much less effective in these products than in culture systems; no significant differences in Listeria counts were observed in matured salami inoculated with MCS or MCS1 bacteriocin-negative strain. However, inoculated Lactobacillus strains prevent L. monocytogenes growth, which confirms the importance of using suitable starter culture for salami maturation.

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