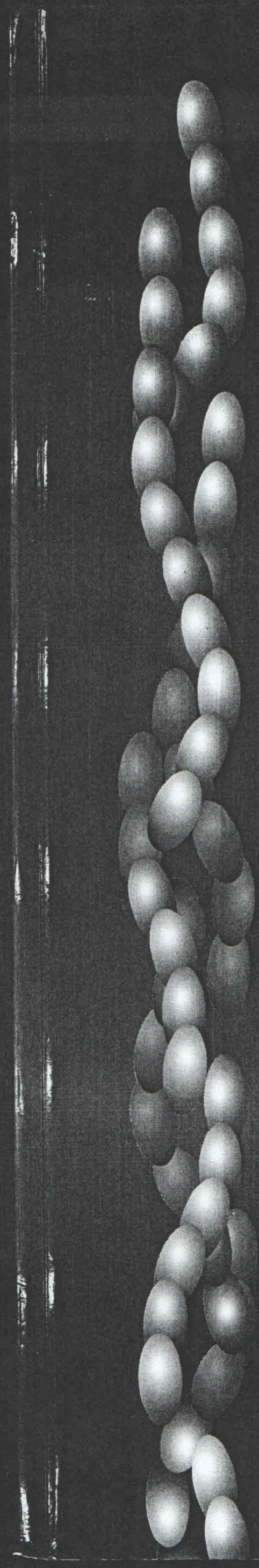

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les bactéries lactiques

Lactic acid bacteria

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BACTERIOCIN-INDUCED LYSIS OF LACTIC ACID BACTERIA

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Abstract

Increasing attention has been focused recently concerning the effect of cell-lysis on the release of intracellular proteolytic enzymes into the cheese matrix and thereby on the ripening process. Previous work in our laboratory has described the characteristics of a bacteriocin able to induce lysis in sensitive strains. The lytic effect of the bacteriocin on *Lactococcus lactis* subsp. *lactis* IFPL359; its Lac$^-$ Prt$^-$ derivative *L.lactis* T1 and *Lactobacillus casei* subsp. *casei* IFPL731, used as starter and adjunct starter in goat's milk cheese making, have been studied with the aim of using it to induce early cell lysis during cheese ripening. Lytic effect of the bacteriocin assayed was maximal against *L. lactis* T1, and high values of lysis were found for this strain during all exponential-phase growth. Lysis response to the bacteriocin seemed to be strain dependent and related to growth conditions.

Materials and methods

Bacterial strains used were from the culture collection of the Instituto del Frío (CSIC), Madrid, Spain. The strains studied were *Lactococcus lactis* subsp. *lactis* IFPL359 and *Lactobacillus casei* subsp. *casei* IFPL731, which have been successfully used in goat's cheese manufacture, as starter and adjunct starter, respectively, and *L. lactis* subsp. *lactis* T1, which is a Lac$^-$ Prt$^-$ strain derived from *L. lactis* subsp. *lactis* IFPL359, and has been used to accelerate flavour development in the manufacture of reduced-fat semihard cheese (Rodríguez, J., Requena, T., Goudédranche, H., Maubois, J.L., Juárez, M. 1996. Accelerated ripening of reduced-fat semi-hard cheese from a mixture of cow's, goat's and ewe's ultrafiltrated milk by using a Lac$^-$ Prt$^-$ strain of lactococci. Lait 76, 513-522). The bacteriocin producer was *Lactobacillus curvatus* IFPL105 which was isolated from raw goat's milk. Preliminary studies of the bacteriocin characterization have been published elsewhere (Casla, D., Requena, T., Gómez, R. 1996. Antimicrobial activity of lactic acid bacteria isolated from goat's milk and artisanal cheeses: characteristics of a bacteriocin produced by *Lactobacillus curvatus* IFPL105. J. Appl. Bacteriol. 81, 35-41).

Bacteriocin (500 AU/ml, final titre) was added to cells of lactococci and lactobacilli suspended in 0.1 M citrate-phosphate buffer, pH 5.5 ($O.D._{650}$ 0.3), after being harvested over their growth in M-17 or MRS broth at 60-min intervals for the first 8 h and after 24 h of growth. Cell suspensions were incubated at 35 °C and the time course of decrease in O.D. was recorded every 60 min. Percentage of lysis was determined as follows: $\%lysis=100-(A_1/A_2 \times 100)$, where A_1 is equal to the lowest and A_2 to the highest O.D. measured during incubation. Induction of cell lysis was also studied in logarithmic phase cultures growing in M-17 or MRS broth and milk at 30 °C, by direct addition of 500 AU/ml bacteriocin to the cultures grown to an $O.D._{560}$ of approx. 1.0, and monitoring of further $O.D._{560}$ decrease at 60-min intervals until the end of the incubation period ($O.D._{560}$ in milk cultures was measured in one-tenth diluted samples using 0.2% EDTA, pH 12.0). Cell lysis was also monitored by determining the activity of the intracellular enzyme post-proline dipeptidyl aminopeptidase and by assessment of changes in $O.D._{260}$ and $O.D._{280}$ for the release of nucleic acids and proteins, respectively.

Results and discussion

High values of lysis were found for *L. lactis* T1 when 500 AU/ml of bacteriocin were added to cell suspensions in buffer after being harvested during all exponential-phase growth. Maximal percentage of lysis (48%) was reached about 3 hours after the beginning of active growth in broth culture. The effect of the inhibitory compound on lysis induction of the other strains was not remarkable.

The results obtained after direct addition of the inhibitory compound to the growth medium indicated that all three strains lysed to a higher extent during active growth in milk than in M-17 or MRS broth. Cell viability in milk cultures also decreased in about 4 log units for lactococci and 3 log units for *L. casei* IFPL731. Once again, *L. lactis* showed higher susceptibility to lysis induction by bacteriocin than *L. casei*, being the strain Lac^- Prt^- the most sensitive one.

The bacteriocin used in this study is of great potential application in the cheese industry, not only by its demonstrated effect against spoilage bacteria or food-borne pathogens but also by its bacteriolytic effect which allows the use of the bacteriocin producer as an adjunct starter to accelerate cheese ripening. The bacteriocin is particularly active against *L. lactis* T1 (Lac^- Prt^-), which has been successfully used to enhance proteolysis and cheese flavour development.