THE AUTOLYTIC BEHAVIOUR OF LACTOCOCCI STRAINS ISOLATED FROM IBORES CHEESE

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INTRODUCTION

Lactococci are widely used as starter bacteria for cheese making, and play a crucial role in the development of the organoleptic quality and microbiological safety of fermented products. During ripening, their cell wall proteinases, together with the rennet enzymes and plasmin, hydrolyse milk caseins into peptides, which are further hydrolysed by the lactococci peptidases. This leads to the formation of small peptides and free amino acids which are flavour precursors, and to the degradation of undesirable bitter peptides.

Starter cell lysis is a necessary step to release the cytoplasmic peptidases in the curd to allow them to reach their substrates. Hence, autolysis of lactic acid bacteria (LAB) plays an important role in flavour development during cheese ripening [1].

In this work, the best autolysis conditions of some lactococci isolated from Ibores cheese were evaluated with the aim of choosing the most suitable strains to be used as autochthonous starter.

MATERIALS Y METHODS.

Bacterial cultures. The microorganisms used in this study were: *Lactococcus lactis* subsp. *lactis* CCBA 119, CCBA 201, CCBA 203, CCBA 208, CCBA 213, CCBA 318, CCBA 321and CCBA 434 obtained from Ibores cheese, and CECT 118 as a control.

Measurement of the rate of autolysis. Lactococcal strains were grown in M17 broth. The cell suspension was set at an optical density of 0.8 to 1.0. The percentage decrease in optical density at 650 nm after incubation for 24 h was used as a measure of the autolytic activity.

Effect of temperature, sodium chloride concentration an pH on the rate of autolysis. Experiments of temperature, sodium chloride concentration and pH were carried out as described by El-Kholy et al. (1998)[2].

Screening of bacteriolytic activity. Agar plates containing 0.2% (wt/vol) autoclaved, lyophilized *Micrococcus lysodeikticus* were incubated for 48 h at 30ºC. Bacteriolytic activity was tested as described by Ostlie et al. (1995)[3].

Detection of lytic activity in SDS-polyacrylamide gels. Lytic activity was detected *in situ* by using SDS-12.5% (wt/vol) poliacrylamide gels containing 0.2% (wt/vol) autoclaved, lyophilized *Micrococcus lysodeikticus* as described by Buist et al. (1995)[4].

RESULTS AND DISCUSSION

The autolytic activity of 8 strains, each from a different phenotypic type, were evidenced by the reduction in optical density of the cultures. Experiments were carried out at different pH values (4.5-7.0), NaCl concentrations (0.0-2.0 M), and temperatures (10-55º C) for 24 h.

Cell lysis of almost all strains was maximal at pH's 6.5 and 7.0, and 40º C, and was highly variable for the different NaCl concentrations. The strains *Lactoccus lactis* subsp. *lactis* CCBA 201, CCBA 203, and CCBA 208 showed (Figure 1) the best behaviour in the sense that the most suitable strains for autochthonous starters sould have both good autolytic activity and satisfactory proteolytic behaviour under traditional ripening conditions.

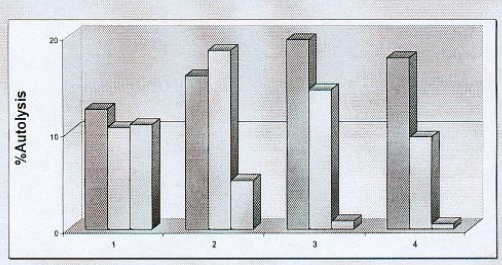

Figure 1. Effect of pH 5.5, temperature of 10ºC and 0.1 M sodium chloride on the autolysis of *Lactococcus lactis* subsp. *Lactis* CCBA 208 (1), CCBA 203 (2), CCBA 201 (3) and CECT 185 (4) as a control.

Lactococcal strains were screened for bacteriolytic activity against *Micrococcus lysodeikticus* on agar plates (Figure 2) [3]. The strains showed differences in lytic activity after incubation period. The hydrolysis zone varied between 1 and 5 mm in diameter.

Figure 2. Lytic zones surrounding *Lactococcus lactis* subsp. *lactis* CCBA 208 grown on M17G agar containing lyophilized *M. Lysodeikticus* cells (0.2%)

We analyzed the bacteriolytic activities of strains *L. lactis* by renaturing SDS-PAGE technique. The substrate was included in the gel, autoclaved *M. lysodeikticus*. The activity bands were identified (Figure 3): band A_1, a major band at 45 kDa which corresponded to major peptidoglycan hydrolase AcmA; band A_0, a minor band at 50 KDa which was found to be a precursor of AcmA; and band A_2, a minor band at 38 Kda which was probably a degradation product of AcmA.

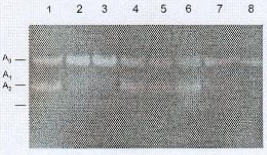

Figure 3. Separation of Lytic enzymes in the supernatantsv of lactococcal strains by SDS-PAGE. Lanes: 1, *L.lactis* subsp. *lactis* CCBA 119; 2, *L.lactis* subsp. *lactis* CCBA 201; 3, *L.lactis* subsp. *lactis* CCBA 203; 4, *L.lactis* subsp. *lactis* CCBA 208; 5, *L.lactis* subsp. *lactis* CCBA 213; 6, *L.lactis* subsp. *lactis* CCBA 321; 7, *L.lactis* subsp. *lactis* CCBA 321; 8, *L.lactis* subsp. *lactis* CCBA 434.

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