Peptides Produced by Selected Lactose-Positive and Lactose-Negative Lactococci in a Model Cheese Ripening System[1]

H. M. ABU-TARBOUSH,[2] R. T. MARSHALL, and H. HEYMANN
Department of Food Science and Nutrition
University of Missouri
Columbia 65211

ABSTRACT

Pasteurized skim milk was placed in a dilution bottle with buffer (pH 5.1 ± .1) that had been immobilized in agar. Rennet and lactococci (10^9/ml) were added. Microbial growth was inhibited with penicillin, natamycin, and nalidixic acid. Following incubation at 25°C for 12 d, proteinase activities were quantified by the *o*-phthaldialdehyde assay and peptides produced were determined by reversed-phase HPLC. Four lactose-negative mutants were among the 14 strains tested. Lactose-negative mutants were significantly less proteolytic than lactose-positive strains with one exception, but all strains were proteolytic. Three of the lactose-positive strains were two to five times more proteolytic than the other seven. Peptide profiles from reversed-phase HPLC were separated by cluster analysis into four groups with similarities greater than .64. The most proteolytic strains, which were lactose-positive, produced numerous hydrophilic peptides with chromatographic retention times of 5 to 25 min, whereas the lactose-negative strains produced numerous hydrophobic peptides with retention times greater than 50 min.

INTRODUCTION

Lactococci possess several proteinases and peptidases that hydrolyze proteins to peptides and amino acids producing desirable cheese flavor (27, 28) and sometimes bitterness (35). Whereas lactose-positive (Lac^+) lactococci possess surface-bound proteinase, lactose-negative (Lac^-) mutants generally possess little or none (15).

Numerous studies on proteinases and peptidases of lactococci have been reported (6, 7, 10, 11, 12, 18, 21, 22, 33, 34, 37), and numerous methods (2, 4, 13, 14, 17, 30) have been used to quantify proteolysis. Several methods exist for extraction of peptides from cheese or protein hydrolysates (19). Extraction with ethanol appears to be most satisfactory (20, 29). Furthermore, HPLC is now available to replace the less satisfactory methods of assaying for peptides and amino acids, e.g., chromatography by ion exchange or gel filtration methods or electrophoresis.

In the present research, the resting cells of 14 strains of lactococci were incubated singly in skim milk that had been treated with the amount of rennet used in cheese making. This proteinaceous substrate was held in a pH range characteristic of Cheddar cheese. As proteolytic activity peaked, samples were analyzed for peptides. The objective was to provide peptide profiles for use in selection of lactococci suitable for use in experiments on accelerated cheese ripening.

MATERIALS AND METHODS

Identities, lactose-fermenting ability, and sources of the 14 cultures studied are shown in Table 1. Twelve were *Lactococcus lactis* ssp. *cremoris* and 2 were *L. lactis* ssp. *lactis* as reported by sources. Other work in this laboratory suggests some of the strains called *L. cremoris* are actually *L. lactis*.

Lactose-positive strains were grown in M17 medium (36), whereas Lac^- strains were grown in modified M17 medium in which glucose was substituted for lactose (MM17). Tubed media were sterilized at 121°C for 15 min. Cultures were transferred weekly during experiments and were propagated at 30°C for 16 h.

Received December 19, 1988.
Accepted June 26, 1989.
[1]Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 10718.
[2]King Saud University, Riyadh, Saudi Arabia.