PROTEINASES OF LACTIC STREPTOCOCCI

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ABSTRACT

^{14}C-Labelled milk proteins and peptides were required for studies on the nitrogen nutrition and proteolytic enzymes of starter bacteria. Therefore, a cow was injected with a mixture of ^{14}C-labelled amino acids (2mCi) and milked at intervals over 28 h. The first milking (3.75 h) contained the most active components with the caseins, β-lactoglobulin and α-lactalbumin having a specific activity of 2.3×10^6 disintegrations per min (dpm) / g while lactose and triglycerides had specific activities of 2.3×10^5 dpm / g and 3.4×10^4 dpm / g, respectively. Thirteen amino acids isolated from acid hydrolyzed milk protein contained radioactivity. Only 6% of the label injected was recovered in milk protein in the first 28 h.

The nitrogen nutrition of *Streptococcus cremoris* AM_2 and E_8 was studied by adding ^{14}C-labelled milk proteins, peptides and single amino acids separately to unlabelled milk and then determining the incorporation of radioactivity into bacterial protein during growth. A specially prepared low heat skim milk powder having a content of potential nitrogen sources similar to that of fresh milk, was reconstituted for use as the growth medium. At low cell densities, free amino acids and peptides were used as nitrogen sources. As the cell density increased, milk protein became an increasingly important nitrogen source and the cells became dependent on their cell wall-bound proteinase. All caseins tested, β-lactoglobulin and α-lactalbumin were used as nitrogen sources by both strains.

A proteinase assay was evaluated in which ^{14}C-labelled casein (2.3×10^6 dpm / g) was used as the substrate. This assay was used to study the spontaneous release of cell wall-associated proteinases from milk-grown cells of lactic streptococci. Eight strains of *S. cremoris* and two strains of *S. lactis* released proteinase when cells were held in buffer. An exception was *S. cremoris* ML_1 which did not release significant activity. The rate of proteinase release increased with rise in temperature (0 to 34^{o}C) and pH (5.5

to 8.7) although inactivation was apparent at 34°C and pH 8.7. With all strains, release of proteinase was suppressed by the addition of $CaCl_2$ to the buffer, by lowering the temperature to 0°C, or by lowering the pH to 5.5. The rate of proteinase release varied markedly with different strains. A possible mechanism for the release of proteinase from the cell wall is discussed.

Cheddar cheese was made with cultures containing different proportions of proteinase-positive (Prt^+) and proteinase-negative (Prt^-) cells. This allowed the level of starter proteinase to be varied while the total concentration of starter cells in the curd at salting was kept constant. Cheeses with 45 to 75% Prt^- cells developed significantly less bitterness than cheeses containing only Prt^+ cells, thus providing direct evidence that the level of starter proteinase has a role in bitterness development in Cheddar cheese. The involvement of starter peptidases in the removal of bitter peptides is discussed.

PREFACE

The manufacture of Cheddar cheese and lactic casein involve the fermentation of milk by lactic streptococci. Since these fermentation industries are of major importance to New Zealand an understanding of the growth and metabolism of lactic streptococci in milk is desirable. This thesis describes research carried out on a number of topics related to the nitrogen metabolism of lactic streptococci. A novel approach adopted in these investigations was the use of radioactive milk, or milk fractions, prepared biosynthetically in the mammary gland of a cow after ^{14}C-labelled amino acids were injected into the bloodstream. The preparation and analysis of this material is described in Section I. Radioactive components were isolated from this milk and used to evaluate the potential nitrogen sources for growth of lactic streptococci in milk (Section II). Radioactive casein was used as substrate in the assay of cell wall-associated proteinases, enzymes which catalyze the first step in the breakdown of milk protein to the free amino acids required for synthesis of bacterial protein. An evaluation of the proteinase assay and studies on the release of proteinase from the cell wall of intact cells, are contained in Section III. Proteolytic enzyme systems of lactic streptococci are not only involved in supplying essential nutrients for cell growth during cheese manufacture but are also active during subsequent cheese ripening. Section IV deals with the effect of the level of cell wall-associated proteinase on bitterness development in Cheddar cheese.

Parts of this thesis have been published in the following papers:

Mills, O E (1976). The preparation of ^{14}C-labelled milk proteins. N Z Jl Dairy Sci. Technol. 11, 164 - 168.

Mills, O E & Thomas, T D (1978). Release of cell wall-associated proteinase(s) from lactic streptococci. N Z Jl Dairy Sci. Technol. 13, 209 - 215.

Mills, O E & Thomas, T D (1980). Bitterness development in

Cheddar cheese: Effect of the level of starter proteinase. N Z Jl Dairy Sci. Technol. 15, 131 - 141.

Mills, O E & Thomas, T D (1981). Nitrogen sources for growth of lactic streptococci in milk. N Z Jl Dairy Sci. Technol. 16 (in press).

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