SUMMARY

Lactic streptococci are widely used in the manufacture of fermented milkproducts, of which cheese is the most important. The increased economic importance of these organisms in the dairy industry and the scaling-up of the fermentations during the last decades has led to an intensification of research in the lactic streptococci. Their prime functions during the manufacture of cheese are a rapid acid production through the conversion of lactose to lactate and the formation of flavourous compounds. Rapid growth, necessary for rapid acid production, is completely dependent on the ability of these auxotrophic organisms to liberate essential amino acids from milkprotein (casein). Moreover, the amino acids and peptides formed by this proteiolysis are important determinants in cheese flavour and -taste.

The observation that the proteolytic activity is an unstable trait in some strains of lactic streptococci was the start of a project aimed at determining the genetical basis of this proteolytic instability and the isolation of the proteinase gene. To be able to do this it was necessary to develop a method to genetically manipulate lactic streptococci. Chapter II describes the development of a host-vector system based on a cryptic Streptococcus cremoris plasmid and a model strain of S. lactis. The system is very versatile because the plasmid-vectors constructed are replicated and expressed in S. lactis, Bacillus subtilis and Escherichia coli. Thus, the advanced recombinant DNA methodologies developed for the latter two organisms have become available for genetical research in lactic streptococci. In Chapter III this was demonstrated by the cloning in B. subtilis of a piece of DNA specifying the proteolytic activity of S. cremoris Wg2. The resulting recombinant plasmid was transferred to a proteolytic deficient strain of S. lactis using the protoplast transformation protocol described in Chapter II. The cloned piece of DNA restored the ability of the S. lactis cells to rapidly grow in milk with concomitant rapid acid production. The gene products specified by the DNA fragment were identified both in B. subtilis and S. lactis with crossed immunoelectrophoresis. This proved that the structural gene for a

proteinase had been cloned. In Chapter IV this was confirmed. In this chapter the results of a nucleotide sequence analysis of the entire DNA fragment are presented. The fragment, 6516 base pairs in length, carries two incomplete open reading frames (ORF's) positioned in opposite direction. The longest ORF was sequenced completely using an overlapping piece of DNA. The complete ORF contained 1902 codons and was large enough to synthesize a protein of 200 kilodaltons (kD). A protein homology comparison revealed that the 200 kD protein shared similarities with the serine proteases of several bacilli, the subtilisins. Especially, three regions which constitute the active centre in the subtilisins are well-conserved in the S. cremoris Wg2 proteinase. This proved that the cloned DNA fragment, specifies a functional serine proteinase. The results in Chapter V show that, in spite of the fact that only 85% of the gene was cloned, both the activity and the specificity of the truncated proteinase were unchanged. An in vitro constructed deletion derivative of the proteinase, lacking the C-terminal 343 amino acids, still had the same specificity. From a mixture of α-, β-, and k-casein, as found in milk, it only degraded the β-casein into several specific polypeptides. A further deletion analysis of the proteinase gene showed that two proteins of the proteolytic system of S. cremoris Wg2, identified immunologically, were both encoded by the proteinase gene. This observation was the basis for the development of a tentative working model for proteinase processing, integrating the genetical and immunological data presented in this thesis, and biochemical and genetical data derived from the literature. The model postulates self-digestion of a large cell wall-bound proteinase as a result of the isolation procedure. This causes the release and purification of a 140 kD truncated proteinase which is found in two immunologically distinct conformations. A working model was developed on the basis of which the characterization of lactic streptococcal proteinases and the classification of S. cremoris strains containing these proteinases was discussed.