The starter cultures used in the Dutch cheese industry are mixed cultures of lactic acid bacteria. The most important bacterial species in these starters is *Streptococcus cremoris*. This species is represented by many different strains that vary strongly in several properties important for cheese manufacture. Some of these properties have been observed to be unstable during cultivation. They can be lost spontaneously by many strains since their genetic information is located on extrachromosomal DNA (plasmids). These spontaneous mutations increase the complexity of the mixed starter cultures. These cultures containing many different strains and variants of *S. cremoris* are the subject of this thesis. Some of the numerous interactions that can occur (see Chapter I) and their effect on the composition of the mixed cultures are described. The proteolytic system is an important feature of the lactic streptococci as it enables growth with concomitant acid production in milk and gives rise to flavour development during the cheese ripening. Special attention has been given in the present investigation to this system. In chapter II the occurrence and population dynamics of protease negative variants was described and Chapters IV and VI are devoted to competition between strains of *S. cremoris* that are known to have different proteolytic systems. In Chapter III an analysis is given of the biochemical differences between these proteolytic systems. In Chapter V a method is described that can be used to identify *S. cremoris* strains and variants occurring in mixed cultures.

Protease negative (Prt⁻) variants arise spontaneously in starter cultures. Under certain conditions these variants become dominant and growth and acidification of these starters in milk are reduced. This process and the methods which can be applied to prevent the Prt⁻ from becoming dominant are described in Chapter II. The Prt⁻ were shown to outcompete the wild-type (Prt⁺) of *S. cremoris* strains Eg, HP and Wg₂ at pH-values higher than 6.0. This process was studied in more detail in *S. cremoris* Eg. At low pH-values the wild-type had a selective advantage. This pH-dependent selection was not found in all media tested. The poor growth of the Prt⁻ variant at low pH was not due to lower internal pH-values. By growing *S. cremoris* Eg and Wg₂ in acidified milk (pH=5.9) the proteolytic activity of the cultures could be stabilized.

In continuous cultures under amino acid-limitation at low dilution rates the Prt⁺ of *S. cremoris* Eg and HP were found to have a selective advantage over the Prt⁻ variants at all pH-values. This was apparently due to lower affinity-constants (Kₗ) of the Prt⁺ for amino acids. A stable high fraction of Prt⁺-variants could be maintained in continuous cultures by using growth media with low concentrations of casein. At high dilution rates (D=0.4) nearly all cells were protease positive.
Before the experiments on the population dynamics of mixtures of different strains of *S. cremoris* (Chapters IV, V and VI) were started the differences in proteolytic systems were first analyzed (Chapter III). This was done by isolating them from the cell-wall and studying them with crossed immunoelectrophoresis (CIE). At least four immunologically different components of the proteolytic systems were found. One of these proteins, protein A, was produced by all strains tested. The proteolytic activity of this enzyme was demonstrated with a zymogram staining technique which is based on the degradation of Coomassie-brilliant blue stainable casein. The CIE-patterns of the different proteolytic systems indicated that each *S. cremoris* strain produces a characteristic combination of proteins. On the basis of these combinations, the different *S. cremoris* strains were classified into four groups. The proteolytic system of *S. cremoris* Wg2 has been studied in more detail. Its two components, protein A and protein B, could not be separated by standard chromatographic techniques since both proteins have identical molecular weights (~140000 Da) and isoelectric points (pI = 4.5). Specific antibodies were raised against proteins A and B by excision of the different immunoprecipitates from CIE-gels and using these as antigens for raising new antibodies. These antibodies were used to specifically remove protein A or B from solutions containing both proteins. In this way the proteins could both be characterized separately. Protein A and B both turned out to be proteases that are inhibited by the serine-protease inhibitor phenylmethylsulfonylfluoride (PMSF). Each protein accounts for ~50% of the total proteolytic activity isolated from *S. cremoris* Wg2. The specific antibodies against the proteases were also used for immunogold labeling studies. The proteases were clearly seen to be located at the outside of the cell wall. When the genetic information for the proteases was cloned and expressed in *Streptococcus lactis* and *Bacillus subtilis* they maintained exactly the same localization.

In Chapter IV the first competition experiments are described of different *S. cremoris* strains in mixed cultures. *S. cremoris* HP was found to grow poorly on agar plates under aerobic conditions in comparison to several other strains of *S. cremoris* (Wg2, ML1, AM1 and Eg). This made it possible to determine the cell numbers of strain HP when grown in mixed culture with other strains under different culture conditions. None of the mixtures was stable in batch culture as a result of differences in the maximum specific growth rate. In continuous culture under lactose limitation strain HP outcompeted strain Eg and ML1 at low dilution rates, but at high dilution rates and in batch culture the reverse was observed. This was due to the fact that the μ-S curves which relate specific growth rate to lactose concentration were crossing.

To enable a study of population dynamics in other and more complex mixtures of *S. cremoris* strains a method had to be developed that could distinguish the various strains specifically in mixed cultures.
The antisera interacted specifically with the corresponding strain in mixtures of up to 9 different *S. cremoris* strains. The antisera can be used for analyzing the composition of mixed cultures containing these strains by immunofluorescence. Competition experiments were performed in batch and continuous cultures under lactose limitation (confirming the results described in Chapter IV) and under amino acid limitation.

A bacteriophage sensitive variant of *S. cremoris* SK11 (SK1129) could be distinguished from a bacteriophage resistant variant (SK1143) by the same immunofluorescence technique. The competition between the two variants and their stability in pure cultures could be followed by applying the specific antibodies.

The immunofluorescence method was also applied with antibodies against the purified proteolytic system of *S. cremoris* Wg2 (see Chapter III) to determine the presence of proteases in several *S. cremoris* strains grown at different culture conditions.

In Chapter VI this immunofluorescence method was used to study the population dynamics of mixtures containing different strains of *S. cremoris* after investigating which environmental factors in milk influence the composition of the mixed cultures. The maximum specific growth rates of several *S. cremoris* strains in milk were found to be 10-40\% lower than in other, nutritious media. The growth rates could be increased by the addition of an amino acid mixture or of extra casein to the milk. When diluted milk was used, the growth rates of the streptococci decreased significantly. This decrease could be prevented by restoring the casein concentration in the diluted milk to its normal value (3\%). This indicated that casein was the growth-limiting substrate in milk. The casein-limitation led to an amino acid-limitation during growth. This could be demonstrated for *S. cremoris* by continuous cultivation at increasing dilution rates in media with low casein concentrations (Chapter VI).

In exponentially growing milk cultures the free amino acids were measured to determine which amino acid(s) was(were) absent and could possibly be growth-limiting in milk. Of these missing amino acids the essential ones (leucine, arginine, phenylalanine, isoleucine, histidine and methionine), together, stimulated the growth of *S. cremoris* in milk cultures. The amino acids leucine and phenylalanine appeared to play major role in this stimulation. These two are, presumably, the first amino acids that become limiting during growth in milk.

The observed growth-limitations in milk made it relevant to study the effects of casein- and amino acid-limitation on the composition of mixed cultures in the chemostat. Differences in apparent affinity-constants of the strains for casein, glutamate and leucine (see Chapter V) were recorded for the strains. This led to different dominancies in the mixed cultures depending on the dilution rate of the chemostat.