Lactococci are generally used as starter cultures for the production of cheese and other dairy products. Two metabolic properties of these bacteria are of primary importance for a successful milk fermentation. These are the conversion of milk sugar (lactose) into lactic acid and the degradation of milk proteins (caseins) to peptides and amino acids. The degradation of caseins is a key process for two reasons; (i) it plays an important role in the flavour development during the process of cheese manufacturing, (ii) it supplies the fastidious lactococci with essential amino acids which enables these bacteria to grow rapidly in milk. The first step in the degradation of casein is performed by a cell wall-associated protease. The released oligopeptides are further hydrolyzed by extracellular peptidases. The subsequent steps involve the translocation of the hydrolysis products (small peptides and amino acids) across the cytoplasmic membrane. Finally, translocated peptides are hydrolyzed by intracellular peptidases which release amino acids for lactococcal biosynthesis.

This thesis describes a study of the translocation of peptides and amino acids across the cytoplasmic membrane of *Lactococcus lactis*. It includes a molecular and bioenergetic study of dipeptide transport and an investigation of the role of peptide transport during growth on casein-containing media. On basis of the results obtained in this study, a model of the process of casein degradation by lactococci was constructed.

Chapter II describes an investigation of the mechanism and energetics of alanyl-glutamate (a dipeptide) transport *L. lactis*. To make a distinction between peptide transport and hydrolysis, a peptidase-free model system was used for the transport assay. This system consists of a hybrid membrane which was constructed by fusion of bacterial membrane vesicles with liposomes containing beef heart cytochrome-*c*-oxidase as a proton motive force (PMF) generating device. The main conclusion of this study is that dipeptide utilization by *L. lactis* takes place by a two-step process (see figure 3, model 3, page 17). The first step is the translocation of the peptide across the cytoplasmic membrane via a specific peptide transport system. The second step is the hydrolysis of the peptide by an intracellularly located peptidase. Furthermore, it is demonstrated that dipeptide transport is driven by the proton motive force (PMF). This study represents the first example of peptide transport in bacterial membrane vesicles.

The role of the lactococcal dipeptide transport system is investigated in Chapter III. For this purpose a dipeptide transport deficient mutant was isolated by selection for resistance to the toxic dipeptide L-alanyl-ß-chloro-L-alanine. This study shows that a functional dipeptide transport system is essential for growth of *L. lactis* on casein-containing media.

In Chapter IV it was demonstrated that, for *L. lactis*, proline-containing dipeptides are nutritionally superior to free proline. The explanation for this was found to be on the level of transport. Both auxotrophic and prototrophic strains of *L. lactis* lack a proline specific uptake system. However, proline-containing peptides can be taken up actively via the PMF-driven peptide transport system described in Chapter II. Since ß-casein is an extremely proline-rich protein, the significance of this conclusion is discussed in the context of the mechanism of casein utilization by lactococci and growth on casein-containing media.

An analysis of the size-restriction and substrate specificity of different peptide transport systems is given in Chapter V. One system was found to transport dipeptides and tripeptides, but not amino acids and oligopeptides (a di-tripeptide transport system). With an alanine/glycine transport mutant and a di-tripeptide transport mutant, the existence of an oligopeptide transport system in *L. lactis* was demonstrated.

Finally, in Chapter VI a detailed description is given of the process of casein degradation by lactococci. In this chapter, the attention is focused on the role of transport systems in this process. The major conclusion of this analysis is that during growth on ß-casein, the essential or growth stimulating amino acids isoleucine, methionine, phenylalanine and proline are supplied exclusively as an X-Pro dipeptide.