Non-fermentation or delayed fermentation (by some strains) of lactose by *Vibrio cholerae* is an important property which is used in clinical laboratories, along with other tests, for the identification of *V. cholerae*. This organism, however, is known to give ONPG (orthonitrophenyl-β-D-galactopyranoside) positive reaction quite readily producing yellow colour in diagnostic assays. Since ONPG is a substrate for the enzyme β-galactosidase, the above observation is indicative of the presence of β-galactosidase in this organism. The reason for this apparently contradictory behavior of *V. cholerae*, the presence of β-galactosidase on one hand and non-fermentation or delayed fermentation of lactose on the other was not known. This thesis presents the results of a study carried out to understand the mechanism underlying this phenomenon and also to characterize in detail the enzyme β-galactosidase of *V. cholerae* which was determined to be present in this organism by first purifying it and then cloning and sequencing its gene.

Experiments performed with three different kinds of indicator plates indicated that substrates for β-galactosidase which can enter cells through diffusion and do not need permease, could be hydrolysed by *V. cholerae* providing strong indication that *V. cholerae* does indeed possess β-galactosidase and is unable to accumulate lactose from the growth medium. It was further found that when *V. cholerae* are forced grown on a medium with lactose as the sole carbon source, it acquired the ability to take up lactose from the medium. It was inferred that wild type *V. cholerae* display lac$^-$ phenotype because they lack a functional permease system for the uptake of lactose.

Confirmation for this conclusion came from two different kinds of experiments:

(i) It was shown that growth of lactose grown *V. cholerae* was inhibited by TONPG (orthonitrophenyl-β-D-thiogalactopyranoside), a metabolic poison which requires active β-galactoside permease for its entry into the cells, while *V. cholerae* grown on media containing a carbon source other than lactose, were indifferent to it.

(ii) It was found that while lactose grown *V. cholerae* could take up ^{14}C-lactose from the medium displaying a "hyperbolic" uptake kinetics, typical for "permease" mediated entry, cells not grown on lactose could not take up ^{14}C-lactose at all. It was found that β-galactosidase in *V. cholerae* is synthesized constitutively. In *E. coli* three enzymes namely β-galactosidase, β-galactoside permease and transacetylase make up the *lac* operon enzyme ensemble; no evidence for the presence of a transacetylase was found in *V. cholerae*.

Hybridization experiments performed with *E. coli lac* operon specific gene probes under reduced stringency conditions demonstrated the existance of a gene homologous to the *lac*Z gene of *E. coli* in *V. cholerae*. However no homolog for *E. coli lac*Y (permease) could be detected in *V. cholerae*, indicating that the "uptake system" acquired by lactose grown cells is distinct from the *lac*Y permease system of *E. coli*.

β-galactosidase from *V. cholerae* was isolated and was found to have a molecular weight of 110 kDa. This enzyme was also immunologically distinct from the *E. coli* enzyme and unlike the *E. coli* enzyme was demonstrated to be active as a monomer. Its physico-chemical properties were studied.

The final chapter of this thesis describes the cloning and partial sequencing of the *V. cholerae* β-galactosidase gene to obtain further information on this system. A 3.1 Kb fragment coding for *V. cholerae* β-galactosidase was cloned from the *V. cholerae* gene library. Through a variety of experiments like *in situ* zymography, and maxi-cell analysis, the authenticity of the clone was confirmed. A

partial sequence covering 271 bases in the 5' end and 294 bases in the 3' end of the gene was obtained, and was compared with the corresponding regions of the *E. coli lacZ* gene both at the base sequence and at the derived amino acid sequence level. The results obtained corroborated the evidence obtained from other experiments that there is a considerable "divergence" in the two enzymes.

In conclusion, it was found that *V. cholerae* has a gene for β-galactosidase in it which codes for a β-galactosidase of molecular weight 110 kDa, which differs from the *E. coli* enzyme in the rather unique way that it is a monomeric enzyme and that its sequence does not perhaps bear a great deal of homology with the *E. coli* enzyme. Even though the enzyme is constitutively expressed in *V. cholerae*, the organism displays a lac^- phenotype because wild type *V. cholerae* lacks a mechanism for taking up lactose from the medium.