# SUMMARY

Small genome size and fast rate of multiplication inside the host have made single stranded DNA phages attractive candidates for studying various processes related to virus multiplication and growth. Discovery and study of the single stranded phages like ϕLf of *Xanthomonas campestris* and VSK and ϕCTX of *V. cholerae* led to the discovery that single stranded phages too are able to lysogenize their hosts by integrating their genomes into the host chromosome. Subsequently, a few other such phages have been discovered. However, besides ϕCTX, the other viruses of this group have not been studied in great detail and there is a paucity of information. This thesis presents the results of our investigation on phage VSKK, a single stranded filamentous vibriophage of type VSK.

Initial characterization of VSKK revealed that its genome comprises a single stranded DNA and its coat structure is made up of a coat protein subunit of molecular weight 4.4 kDa. Unlike VSK, VSKK was found to be released from its host cells spontaneously. The results of experiments, which were initially designed to locate the integration site of this phage, indicated that during lysogenization, the phage genome possibly integrated into an extrachromosomal element, rather than into the host chromosomes. Using Pulsed Field Gel Electrophoresis and Southern hybridization technique it was proved decisively that VSKK lysogenizes its host by integrating into a megaplasmid of about 150 kb in size. To the best of our knowledge, this is the first report of this mode of lysogenization by any phage (chapter 3).

Lysogenization by VSKK of *V. cholerae* BO2, an indicator strain, was found to bring about several changes in the host; e.g. change in fatty acid profile of the cells, colour of the cells etc. Rabbit-ileal loop assay indicated that the phage lysogenization enhances the level of virulence of *V. cholerae* BO2. An increased fluid accumulation was observed in the ileal loops injected with the phage-infected cells compared to the control (chapter 3).

The phage genome was sequenced and found to contain 6834 nt with a G+C content of 43.5%. By *in silico* analyses, ORFs coding for the coat protein (ORF82) and a Zot (Zonula occludence toxin) like protein of ϕCTX and Gene I protein of single stranded coliphages, which are involved in the phage assembly, were detected. The presence of Gene II, designated as ORF367, which is a homologue of REP family of proteins involved in rolling circle replication, could also be identified. Based on multiple alignments, hydropathy plots, secondary structure prediction, charge density and locations of putative ORFs on the VSKK genome two ORFs, namely ORF81 and ORF 184 could be identified as the homologues of Gene V (single stranded DNA binding protein) and Gene III (minor coat protein) of the single stranded phages respectively. The comparison of gene order of VSKK with other phages indicated the possibility of the ORF112 being a functional equivalent of gene X, the product of which is involved in viral strand synthesis (chapter 4).

The presence of a stem loop structure with a putative binding site for replication initiator protein, a conserved nicking site and also a IHF binding site on the viral strand together with the presence of a gene II (ORF367) which could code for a REP like protein, led to the conjecture that the genome of VSKK probably replicates through the rolling circle mechanism. Presence of the stem loop structure with the properties described, on the 2.5 kb *Sau*3AI fragment spanning from nt 539 to nt 680 indicated that the origin of replication could be located on this fragment. By primer extension of replication intermediate molecules and by two-dimensional DNA gel analysis, it was confirmed that the "plus" strand origin of replication of VSKK is located on the 2.5 kb [illegible] fragment of the RF and that this phage indeed replicates by rolling circle mechanism (chapter5).