Conclusions

From the present study following conclusion have been drawn:

- The size of the plasmid pCP289 of *Pediococcus acidilactici* MTCC 5101 is very large; hence for the development of the expression vector, a multi fragment construct, constituted of ped⁺ operon with its promoter and ori region has been obtained from pCP289.

- The Ped operon and ori region fragments were amplified from the plasmid pCP289 using information available in the literature regarding the sequence of plasmid pSMB74 of *Pediococcus acidilactici* H. Insertion site was designated from the restriction sites constituent of the ped operon region, as multiple cloning sequences.

- Expression vector pPBP5 was developed by ligating these two plasmid fragments containing ori and ped⁺ regions and it was characterised for pediocin secretion, by transforming plasmid free *P. acidilactici* MTCC 5101.

- Application of developed expression vector was carried out by cloning a clotting protein fragment into the developed plasmid vector pPBP5. mRNA sequence of gamma chain of human fibrinogen was obtained from NCBI with accession no. NP_068656 (http://www.ncbi.nlm.nih.gov/).
The Fibrin Polymerization Pocket (FPP) sequence was curated from the fibrinogen gamma chain sequence. FPP region of the fibrinogen gamma chain was selected as it is involved in covalent cross linking of fibrin chains to form the clot.

The human mRNA sequence was reverse transcribed and designed into a cDNA as per codon preference of *P. acidilactici* and insertion site of pPB5. The R-DNA thus generated was cloned in *P. acidilactici* MTCC 5101.

The FPP was expressed in *P. acidilactici* and purified using his-tag present in the protein on a Ni$^{2+}$-NTA affinity column. The purified FPP was then characterized by analysis on SDS-PAGE.

The study successfully prepared the expression vector based on ped$^+$ plasmid and cloned the FPP protein as proof of the functionality of the vector in *P. acidilactici* MTCC 5101.