CHAPTER 7

SUMMARY AND CONCLUSION
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- In this study, pediocin PA-1 like bacteriocin producing LAB were isolated from various sources and were characterized by physiological, biochemical and molecular typing techniques including RAPD and RFLP PCR, and subsequently sequencing of the 16S rDNA, and the cultures were deposited at NCIM, Pune.

- Production of pediocin PA-1 like bacteriocin by *Streptococcus equinus* NCIM 5418 from milk origin and *Enterococcus faecium* NCIM 5423 and *Lactobacillus plantarum* Acr2 from vegetable origin are new findings reported in this study.

- In case of *Pediococcus acidilactici* the MGEs like *repB* and tyrosine recombinase (integrase) present in the flanking regions of pediocin plasmid are found to be responsible for DNA transfer of pediocin plasmid among LAB.

- Insertion element (IS*Lpl1*) and mobilization region in the *Enterococcus faecium* NCIM 5423 and *Lactobacillus plantarum* Acr2, in the flanking regions of the pediocin operon plasmid was detected that play a major role in the integration of the pediocin operon from other bacteria.

- The phylogenetic tree of the MGEs found in the intergeneric/interspecific pediocin producers, displays the close association of these genes with the *Lactobacillus* sp. associated with bacteriocin production, antibiotic resistance, etc.

- The native isolates *Ent. faecium* NCIM 5423 and *Lact. plantarum* Acr2 were able to ferment soymilk and maintained their viability for a period of 15 days during storage at 4 °C. Under such in situ conditions the pediocin production was observed by these intergeneric bacteriocin producing LAB.

- The co-cultivation of *Ent. faecium* NCIM 5423 and *Lact. plantarum* Acr2 individually with *Listeria monocytogenes* ScottA during fermentation of soymilk, reduced the *Listeria* count by 2 logs within 6 and 8 h, respectively.

- The quality parameters and sensory score for the *Ent. faecium* NCIM 5423 fermented soymilk was found to be acceptable.
The *in vitro* and *in situ* conjugal transfer of pediocin in soymilk from donors (*Ped. acidilactici* NCIM 5424 and *Ent. faecium* NCIM 5423) to the recipient (*Ent. faecalis* JH2-2) was observed and for the first time the *in situ* conjugal transfer of pediocin PA-1 like bacteriocin in soymilk model with higher transfer efficiency was determined.

The transconjugants obtained were able to produce pediocin. Southern hybridization of the immunity gene suggests the possibility of acquiring the pediocin under *in vitro* and *in situ* mating conditions. Different environmental conditions like temperature and pH could be the reason that affected the production of bacteriocin in transconjugants.

Such intergeneric pediocin producers can be used in the development of industrially important starter cultures, and can act as a bio-preservative and bio-protective culture in the fermentation of dairy and meat products.
FUTURE PERSPECTIVE

This study was conducted inorder to investigate the natural spread of pediocin PA-1 among LAB isolated from different fermented products. Results of this study clearly indicated pediocin–like bacteriocin production among *Ent. faecium* NCIM 5423 and *Strep. equinus* NCIM 5418. However, to study the distribution of the bacteriocin among other genera of LAB, more number of samples are required for further investigation.

The pediocin production by *Strep. equinus* NCIM 5418 and *Lact. plantarum* Acr2 was found to be comparatively low under environmental conditions. Therefore, there is a need to investigate and optimize the cultural conditions for maximum production of bacteriocin in the new isolates reported in the study.

The flanking regions analysed showed several interesting IS elements responsible for the pediocin transfer. However, there is a possibility for the identification of novel MGEs by sequencing the plasmid reported in the present investigation.

The use of *Ent. faecium* NCIM 5423 as a starter culture in soymilk fermentation resulted in obtaining a desirable quality product with anti-listerial activity. Hence this process can be scaled up further.

Further investigation of the plasmid modification and integration of the pediocin encoded plasmids in the transconjugants is required.