



FUTURE PERSPECTIVE

The thesis presents a detailed survey of class IIa bacteriocins with greater emphasis on anti-listerial bacteriocins, and pediocin PA-1 type in particular. Among the many bacteriocinogenic strains tested, strains C40 and C20 (PH-1) were found to be potent against most of the indicator strains used. The strain C40 identified as *Lactobacillus casei* ssp. *casei* had plasmid linked bacteriocin production and carbohydrate fermentation features. Majority of the intestinal isolates were found to be resistant to several antibiotics as compared to the LAB isolated from mushroom fermentation.

The strain C20 identified as *Enterococcus faecium* PH-1, a colonic resident, posses a strong anti-listerial bacteriocin production, had properties of bile salt, temperature and pH tolerance and hence can be exploited as probiotic culture. The rRNA and *tuf* gene sequences reported for identification and characterization of *Ent. faecium* PH-1 was found to be a valuable tool for identification and characterization of new species of bacteria, whose species determination is often cumbersome and complicated.

The intergeneric pediocin PA-1 production by *Ent. faecium* PH-1 was reported. This is the first report for the production of pediocin PA-1 by a bacterium which can survive at extremes of pH, temperature and salt concentrations etc. The megaplasmid encoding bacteriocin production reported in this study is unusual in nature, especially its molecular size. Nucleotide sequencing of the flanking region of the pediocin operon will establish the possible mechanism that took place in acquiring intergeneric pediocin production. Further characterization of sugar fermenting enzymes could help to understand the nature of these enzymes due to their unusual source.

Since strain PH-1 had an lactose hydrolyzing ability, the WP media was designed and the production of pediocin PA-1 was studied. This food-grade medium was found to be economical for the production of pediocin.

Further scale-up studies should be made by different fermentation trials. Further advanced methods of mathematical modeling should be evaluated for maximal production of pediocin PA-1. Isolation and molecular characterization of full length β -gal encoding genes find its application in basic studies. The native strain of PH-1 can even be employed for the commercial production of β -Gal due to its food-grade property and growth at high temperature. For studying the effectiveness of pediocin PA-1 in Indian food-systems, predictive models should be developed and its efficacy against various pathogenic bacteria should be evaluated.

Use of *Ent. faecium* PH-1 as a starter culture or co-culture cum bioprotective culture in vegetable and dairy product fermentation could be exploited since this culture has an ability to utilize melibiose, raffinose and lactose; the principal carbon sources in these food commodities.

The gene coding for the pediocin precursor and its immunity counterpart was cloned and expressed as a fusion protein in *E. coli*. The results showed that high level of pediocin was found in IBs of *E. coli* and its subsequent isolation, purification, solubilization and *in vitro* refolding yielded a biological active pediocin. Low levels of expression of immunity gene was observed upon cloning in pQE vector system having C- and N-terminal tagging. Further scale-up studies for the production of recombinant pediocin and optimization of *in vitro* refolding parameters are needed to obtain quantifiable yield. The recombinant pediocin and its immunity counterpart produced in *E. coli* could be a suitable material for further biophysical and biochemical investigations.