CHAPTER 9

SUMMARY
\textit{Aeromonas hydrophila} cause widespread disease in freshwater fishes like carp, tilapia, eels, milk fish, channel cat fish and produces stress related diseases in salmonids with the common symptoms of ulcerations, exophthalma abdominal distension. Treatment with antibiotics has led to the emergence of resistance strains. The present study was undertaken to identify, characterize the pathogenic \textit{A. hydrophila} and to investigate the role of immunostimulants and probiotics in common carp \textit{Cyprinus carpio} and chichilid \textit{Tilapia mossambicus}.

1. Approximately 114 isolates were obtained from the samples collected from the different fish farms situated in Southern India (Tamilnadu, Kerala and Pondicherry). All the isolates were identified as \textit{Aeromonas} sp. based on the standard biochemical test.

2. For further characterization and rapid identification of pathogenic \textit{A. hydrophila} a PCR assay and immunodiagnosis were developed. Primers for the two genes \(\beta\)-haemolysin (683 bp) and serine protease gene (350 bp) were designed based on the published sequences and amplified using PCR. As many as 27 strains were showing positive amplification of serine protease gene and haemolysin gene.
3. Antisera for β-haemolysin the ECP of *A. hydrophila* were raised in albino rabbit and used for western blot analysis and 24 strains were found to be positive pathogenic *A. hydrophila*. The pathogenicity of these strains was confirmed by injecting into the fish common carp and tilapia.

4. To study the effect of dietary supplements chitin, chitosan, levamisole on immune response of fish and against *A. hydrophila* and *T. mossambicus* were administered with the dietary supplements for 60 days. The non-specific immune response parameters WBC count, NBT and plasma Lysozyme activity were monitored during the experimental period. The higher NBT reduction was observed in chitosan fed fish on day 30 (1.2 ± 0.024 OD). The significant decrease of NBT reduction was observed on day 45 and 60 in all the immunostimulants fed group as compared to control. In respect of WBC count there were no significant differences among the experimental groups during 15th and 30th days except levamisole fed group. The increased number of WBC count was observed on 30th (1.96 ± 0.32 X 10⁴) and 45th day (1.89 ± 0.29 X 10⁴). The significant increase of serum Lysozyme activity was observed on day 15 in the entire immunostimulant fed group than control. The highest serum Lysozyme activity was observed on 30th day of chitosan and chitin fed fish. Immunomodulatory activity of immunstimulants was strongly confirmed by high RPS of *T. mossambicus* fed with chitosan (77.8%), chitin (55.56%) and levamisole (66.67%) on day 60 in the experimental groups.
5. To find out the effect of the immunostimulants on fish in field conditions common carp *C. carpio* was selected and reared in earthen ponds. The fish were fed with the dietary supplements chitin, chitosan and levamisole along with feed, growth and immune response was monitored for 90 days. Fish fed with chitosan no significant difference was observed in all the experiments except chitin fed fish on day 60 (3.42 ± 0.29 X 10^4) and 90 (3.32 ± 0.29 X 10^4) compared with control (2.89 ± 0.14 X 10^4) and (2.97 ± 0.22 X 10^4). Regarding NBT reduction the highest activity was observed on 30th day administration of chitosan (1.128 ± 0.081 OD) and chitin (0.639 ± 0.032 OD). A significant decrease of NBT reduction was observed from 60th day onwards in all the groups compared with control. With respect to serum Lysozyme activity a significant difference was observed in all the experiments compared with control. In all the treatment, the highest activity was observed on 30th and 60th day in chitosan fed fish, followed by chitin fed fish. Fish fed with chitosan (94.9 ± 9.4 g) and levamisole (93.3 ± 8.4 g) recorded better growth than the control (43.5 ± 4.7 g) but decreased growth in chitin fed fish (43.5 ± 4.7 g).

6. To find out the role of other immunostimulants like β-Glucan which is commercially available and Brewers yeast, common carp *C. carpio* were administered with these substances and the non-specific immune response were monitored for duration of 60 days. Like other immunostimulants β-Glucan and brewers yeasts stimulated the immune response and enhanced the survival of fish challenged with *A. hydrophila*. The NBT and Lysosyme activity were maximum on 30th day in β-Glucan (2.0757 ± 0.025 OD; 6228 ± 498) whereas in brewers yeast treatment the maximum immune activity was found on 60th day.
Administration of β-Glucan (20%) reduced the mortality better than brewers yeast (40%) on 30th day challenge. However on 60th day challenge the mortality slightly increased in β-Glucan treatment (25%) whereas brewers reduced the mortality to 30%.

7. A total of 160 numbers of lactic acid bacteria have been isolated from the intestines of *Mugil cephalus* (MC), *Lates calcarifer* (LC) and *Penaeus indicus* (PI). Among these, 16 isolates were showing antagonistic activity against *A. hydrophila*. Finally, three isolates (MC13, LC149 and PI80) and one standard strain (*Carnobacterium divergens*) have been selected for the in vivo and inviter study. All the four strains tested positive for bacteriocin activity. The ECP of the four strains were extracted and analyzed in SDS-PAGE. Compare with in vitro growth in fish gut mucus, all probiotic bacteria were grew well in fish mucus but the growth was less when compare to pathogens (*A. hydrophila* and *V. vulnificus*).

8. Bacterial strains viz. MC13 (isolated from *M. cephalus*), LC149 (isolated from *L. calcarifer*), PI80 and *C. divergens* were fed to along with feed to common carp *C. carpio*. Among the four probiotic strains higher count of bacteria in the intestine of fish was found in PI80 treatment. In this study the enhancement of neutrophil activity was observed in all the strains but the higher activity was observed in MC 13 fed fish (1.525 ± 0.037 OD). The relative percentage survival increased on both challenges on 30th and 60th day. Among the four strains the highest relative percentage survival (78%) was observed on 60th day in MC 13 strain.