The thesis deals with the prevalence and distribution of motile aeromonads in selected ornamental fishes. It also gives an account of the production of extracellular virulence factors and the antibiogram of the different species of motile aeromonads isolated. The growth characteristics and virulence potential of a representative strain of *Aeromonas hydrophila* is also studied. The nucleotide sequencing of the strain was carried out and sequences deposited in Genbank. Survival and immune response of *Cyprinus carpio* under different stress conditions and on probiotic treatment with *Bacillus* NL110, when challenged with *A. hydrophila* is also dealt within this thesis.

Salient findings of the study are summarized as follows

- Motile aeromonads were isolated from 74% of *Poecilia sphenops* and 68% of *Poecilia reticulata* samples collected from ornamental fish farm. They were isolated from different body parts of fish samples such as body surface, gill and intestine. In *P. sphenops*, body surface showed greater prevalence (39.06%), while in *P. reticulata*, intestine showed greater prevalence (35.8%). One
hundred and forty five isolates were characterized to species level. *Aeromonas sobria* was the predominant species (29%) and *A. jandaei*, the second dominant sp. (20%) in both fish samples.

- Motile aeromonads were isolated from 68% of the water samples collected. One hundred and fifty six isolates from the samples were characterized to species level. *A. sobria* was the predominant species in water samples also (34.61%) and *A. trota* was the second dominant species (23.71%).

- In samples from retail aquaria, motile aeromonads were isolated from 84% of *Poecilia sphenops* and 80% of *Poecilia reticulata* samples collected. In both *P. sphenops* and *P. reticulata* samples, gills showed greater prevalence; 40.86 and 36.58% respectively. One hundred and seventy five isolates from the samples were characterized to species level. *Aeromonas sobria* was the predominant species (40.57%) and *A. caviae*, the second dominant sp. (31.43%).

- Motile aeromonads were isolated from 84% of the water samples collected. One hundred and eighty two isolates from the samples were characterized to species level. *A. sobria* was the predominant species (34.80%) and *A. caviae*, the second dominant species in water samples also.

- The k-dominance curve of ornamental fish samples from farm and retail aquaria showed that samples from retail aquaria exhibited less diversity and high dominance contributed mainly by *A. sobria* and *A. caviae*. 


The k-dominance curve of water samples showed that samples from farm exhibited high dominance, contributed mainly by *A. sobria* and *A. trota*.

There was an extensive production of extracellular virulence factors such as gelatinase (100%), DNase (100%), caseinase (>80%) and lipase (>90%) among the motile aeromonads.

β-haemolytic activity was also widespread among the isolates, with all the isolates of *A. hydrophila* and *A. sobria* haemolytic. *A. caviae* was found to be relatively less haemolytic.

No significant variation (*p* >0.05) was observed in the production of extracellular virulence factors in motile aeromonads from farm and retail aquarium vendors.

All the isolates from fish and water samples (both farm and retail aquaria) exhibited resistance to amoxicillin. All the isolates (both fish and water samples) from fish farm were sensitive to ceftazidime, chloramphenicol, ciprofloxacin and gentamicin.

All the isolates (both fish and water samples) from retail aquaria were sensitive to chloramphenicol, ciprofloxacin and gentamicin. All the isolates in water samples were also sensitive to nitrofurantoin.

Number of motile aeromonads exhibiting resistance to nalidixic acid, streptomycin and trimethoprim was found to be significantly higher (*p*<0.05) in fish samples from retail aquaria, compared to that of farm.
In water samples, significantly higher ($p<0.05$) resistance towards streptomycin was exhibited by motile aeromonads in samples from retail aquaria.

Isolates exhibiting resistance to 7 or more antibiotics (MAR index values greater than 0.43) was seen only in fish samples from commercial aquarium vendors. The MAR index ranged from 0.21 to 0.43 in isolates from farm and 0.21 to 0.57 in isolates from aquarium vendors.

In water samples, resistance to 6 or more antibiotics (MAR index value greater than 0.36) was seen only in isolates from commercial aquarium vendors. The MAR index value ranged from 0.21 to 0.36 in samples from farm and 0.21 to 0.57 in isolates from aquarium vendors.

The species *A. hydrophila* exhibited a wide production of virulence factors and MAR indexing revealed that this species is resistant to higher number of antibiotics; therefore a representative strain of *A. hydrophila* identified by biochemical methods was used for further studies and has been confirmed to be the same by 16S rRNA gene sequencing (GenBank Accession No. JX987236).

The strain was found to grow over a wide range of temperature (10-45°C), pH (5-10) and salinity (0-4.5%).

Virulence genes-*aerolysin* (416bp), *haemolysin* (597 bp) and *cytotoxin* (232 bp) were detected in the isolate and LD$_{50}$ of the isolate was found to be $10^{6.1}$cfu/ml.
Physico-chemical analysis of water samples from farm and retail aquarium vendors revealed higher concentrations of ammonia and nitrite in samples from commercial aquarium vendors compared to the farm. The concentration of these factors in retail aquaria were also found to be above the optimal range for ornamental fish culture. In retail aquaria fishes are often stocked at higher densities sometimes with several species in the same tank. Therefore these factors were considered as stress factors in further studies.

- LC$_{50}$ (96-h) of un-ionized ammonia (UIA)-N for *Cyprinus carpio* was found to be 2.05 mg/L. Survival (%) of *C. carpio* reduced from 75% (control) to 58.33 and 45.83 in the 1/10 and 1/5 of the LC$_{50}$ values respectively of UIA-N when challenged with *A. hydrophila*.

- LC$_{50}$ (96-h) of nitrite-N for *C. carpio* was found to be 46.85 mg/L. Survival (%) of *C. carpio* reduced from 79% (control) to 66.66 and 50 in the 1/10 and 1/5 of the LC$_{50}$ values respectively of nitrite-N, when challenged with *A. hydrophila*.

- With increasing stocking densities of *C. carpio*, 12, 18, 24 and 30 fishes in 10 litre water, survival (%) reduced from 72.2 to 64.81, 51.38 and 38.88% respectively when challenged with *A. hydrophila*.

- Significant reduction (*p*<0.05) in immune response of *C. carpio* was also observed in comparison to the control and with increasing concentration of UIA-N, nitrite-N and stocking densities when challenged with *A. hydrophila*, as evidenced from pathogen clearance efficiency of blood and serum bactericidal efficiency.
Total leucocyte count was found to increase significantly with increasing concentration of stress factors and severity of infection. A significant reduction in the lymphocyte count (%) and increase in the neutrophil count (%) was also observed.

Histopathological analysis revealed increasing severity of pathological changes in the gill tissues with increasing dose of stress factors.

Of the three stress factors studied, un-ionized ammonia (UIA)-N was found to be more harmful to \textit{C. carpio} in terms of survival, immune response and histopathological changes.

On probiotic treatment with \textit{Bacillus} NL110, significant increase in survival \((p<0.05)\) was observed in \textit{C. carpio} challenged with \textit{A. hydrophila} compared to the control. Relative percentage survival was found to be 59%.

Bactericidal efficiency of serum was also significantly \((p<0.05)\) higher in the probiotic treated group compared to the control.

Histopathological analysis of gill, liver and intestinal tissues of \textit{C. carpio} revealed protective effect of probiotic \textit{Bacillus} NL110 on challenge with \textit{A. hydrophila}. 

\text{-----SOCR-----}