

ABSTRACT

Molecular identification and pathogenicity study of the *Klebsiella pneumoniae* isolated from fishes of West Bengal

Fish health mainly depends upon the environmental conditions as well as on the farming techniques. However, in present aquaculture scenario farmers are over-stocking their aquaculture farms as well as they are providing excessive feed. The unutilized feed is detrimental to water quality leading to the outbreak of various kinds of diseases. Bacterial infections in farmed fishes due to *Klebsiella pneumoniae* was not reported earlier. The thesis focuses on the molecular identification and pathogenicity study of *Klebsiella pneumoniae* in fresh water fishes. Infected fish samples viz. *Labeo rohita*, *Oreochromis niloticus*, *Labeo catla*, *Cirrhinus mrigala*, *Anabas testudineus* and *Clarias batrachus* were collected from three different districts of West Bengal viz. North 24 Parganas, Nadia, both east and west Burdwan. Bacterial strains were isolated from liver, kidney and blood tissues of the infected fishes. Primarily bacterial strains were identified using biochemical test and further 16S rRNA gene amplification was carried out for the molecular identification of the isolates. The amplified products were sequenced and the sequence was submitted to NCBI. Phylogenetic analysis of the isolated strains revealed the species diversity and showed dispersion of bacterial strains to different location. Antibiotic sensitivity assay was carried out to determine the multiple antibiotic resistance index of the isolated strains. The MAR index was found to be 0.476 which showed that the strains were highly exposed to antibiotics. The LD₅₀ value was found to be 1.05 X 10⁶ CFU /fish. Histopathology of the liver tissue revealed necrosis, vacuolation and disruption of hepatocytes whereas ultra-structural alterations in the renal tubules and glomeruli were evident in the kidney tissue. The bacteria were found to be β-hemolytic positive in nature as they have caused complete lysis of sheep blood on

blood agar plates. Among the array of virulent genes screened the isolates were found to be positive for *fimA*, *fimH*, *mrkA*, *urea*, *ugeA*, *wabG*, *ecpRAB* and *entB*. The amplified genes were sequenced and the sequences were submitted to NCBI.

Various specific and nonspecific immune parameters of the challenged fishes were studied at various time intervals to find out the immune response against this particular pathogen. An elevated myeloperoxidase activity, superoxide production, and lysozyme activity were observed. However, the antiprotease and α -2 macroglobulin activities were found to be downregulated. Alteration of immune related genes *viz.* C3, IL-1 β and IL-6 were also studied in the internal tissues like kidney, muscle and liver. Early expression of C3, IL-6 and IL-1 β gene was observed in the liver tissue however; late expression of all the genes were recorded in the kidney tissue.

The thesis provides a detailed report of *Klebsiella pneumoniae* infection in farmed fishes and also it could be concluded that, *Klebsiella pneumoniae* is an emerging pathogen which could cause huge loss in aquaculture industry. A stringent surveillance is required to identify the abundance of *Klebsiella pneumoniae* in aquaculture farms.

Key words: 16S rRNA gene PCR, fish diseases, genetic diversity, *Klebsiella pneumoniae*, PCR ribotyping, Real Time PCR.