

List of Figures

Chapter 2	
Figure 2.1	: Map showing infection cause by <i>V. parahaemolyticus</i> globally. Different countries are labeled with different color. Map prepare by using Web tool (https://mapchart.net/world.html).
Chapter 4	
Figure 4.1	: Map showing the different geographical locations of sampling sites in the states of West Bengal Andhra Pradesh and Gujarat. Different color (Pink, Red and Green) within different states represented the different district from where sample had been collected.
Figure 4.2	: Schematic diagram of bacteria culture from shrimp and plating in TCBS plate (A). <i>V. parahaemolyticus</i> stick in different media and different color colonies appear in different media (B).
Figure 4.3	: Solid Phase hemolysin assay using 5 % human blood.
Figure 4.4	: PCR amplified products of 16S rRNA of <i>V. parahaemolyticus</i> . Lane 1-7 Isolated <i>V. parahaemolyticus</i> ; Lane M: Molecular weight marker (100 bp DNA Ladder).
Figure 4.5	: PCR amplified products of <i>toxR</i> of <i>V. parahaemolyticus</i> . Lane 1-7 Isolated <i>V. parahaemolyticus</i> ; Lane M: Molecular weight marker (100 bp DNA Ladder).
Chapter 5	
Figure 5.1	: Agarose gel electrophoresis of the PCR products of different virulent genes amplified from <i>V. parahaemolyticus</i> . <i>trh</i> gene (~490 bp) (A); <i>tdh</i> gene (~250 bp) (B); <i>tlh</i> gene (~450 bp) (C); <i>toxR</i> (~368 bp) (D); <i>vcrD1</i> (~493 bp) (E); <i>vp1680</i> (~502 bp) (F); <i>vopD1</i> gene (~515 bp) (G); <i>vcrD2</i> (~300 bp) (H); <i>vopD2</i> (~476 bp) (I); <i>vopB2</i> (~527 bp) (J). M: Molecular weight marker (100 bp DNA Ladder); Lane 1-4 isolated <i>V. parahaemolyticus</i> .
Figure 5.2	: The kinetics of human RBC haemolysis by three different strains of <i>V. parahaemolyticus</i> containing <i>tdh</i> , <i>trh</i> and <i>tlh</i> genes in liquid-phase hemolysin assay.
Figure 5.3.	: (A to H) HEK cells stained with EtBr and Acridine Orange to show the cytotoxicity after 5 h of infection with <i>V. parahaemolyticus</i> . (A and E) Un-infected HEK cell control; (B and F) HEK cell infected with the <i>tdh</i> -positive stain (S24P132); (C and G) HEK cell infected with <i>trh</i> -positive strain (AP429); (D and H) HEK cell infected with the <i>tlh</i> -positive strain stain (SPEM2).
Chapter 6	
Figure 6.1	: Evolutionary relationships among biotypes of <i>V. parahaemolyticus</i> collected from three different states of India.
Figure 6.2	: Mismatch distribution analysis of 183 numbers of 16S rRNA sequences of <i>V. parahaemolyticus</i> from isolated from West Bengal, Andhra Pradesh and Gujarat showing the observed and expected pairwise differences under the sudden population expansion model.

Figure 6.3	: Neighbor-Joining tree of 36 concatenated sequences of four housekeeping genes of <i>V. parahaemolyticus</i> isolated from shrimp farms of India. Different colors represent different clusters.
Chapter 7	
Figure 7.1	: Schematic diagram of detail protocol used in secretomics study of <i>V. parahaemolyticus</i> .
Figure 7.2	: 12% SDS-PAGE profile extracellular proteins of Control (TSB + 2%NaCl) Treated (TSB + 2%NaCl+ Bile salt). Red arrows indicate the bands differentially expressed protein band in control and treated sample.
Figure 7.3	: Representative 2D gels of extracellular protein of <i>V. parahaemoliticus</i> (a) Control (TSB + 2 % NaCl) (b) Treated (TSB + 2 % NaCl + Bile salt). Red arrows indicate the differentially expressed protein spots in control and treated sample. The red circle represents the protein spots present only in the treated sample.
Figure 7.4	: 2D Gel showing the protein spots used for the MALDI-TOF MS/MS analysis.
Chapter 8	
Figure 8.1	: Schematic diagram of RNA isolation from different tissue using Trizol.
Figure 8.2	: Schematic diagram of challenge study (A) and clinical signs appear in the fish after experimental challenge with <i>V. parahaemolyticus</i> .
Figure 8.3	: Cumulative mortality curves for the determination of LD ₅₀ values in Indian Major Carps challenged with <i>V. parahaemolyticus</i> by intraperitoneal injection at different concentrations. [A] <i>tdh</i> positive stain (S24P132) and [B] <i>trh</i> positive stain (AP429).
Figure 8.4	: Histopathological examination of tissues of <i>Labeo rohita</i> infected with <i>tdh</i> -positive <i>V. parahaemolyticus</i> (S24P132) through intraperitoneal injection. [A-B] Photomicrograph of muscle of <i>Labeo rohita</i> [A]: Infiltration of the lymphocyte (L), Myonecrosis (Z). [B]: Infiltration of the lymphocyte (L). [C-D] Photomicrograph of liver of <i>Labeo rohita</i> [C]: Infiltration of the blood cells (Y), Degeneration of the hepatocytes (\$). [D]: Vacuole formation in the liver (&). [E-F] Photomicrograph of kidney of <i>Labeo rohita</i> . [E]: Glomerular degeneration (X), Necrosis of Bowman's capsule (#). [F]: Necrosis of the renal tubules (*), Sclerosis of glomerulus with atrophic tubules (z). [G] Photomicrograph of intestine of <i>Labeo rohita</i> . [G]: Intestinal mucosal epithelial cell falling off (↓) and intestinal bleeding (↓).

Figure 8.5	: [A-B] Photomicrograph of Muscle of <i>Labeo rohita</i> challenged with <i>trh</i> -positive <i>V. parahaemolyticus</i> [A]: Myonecrosis (#). [B]: Infiltration of the lymphocyte (X). [C-D] Photomicrograph of Liver of <i>Labeo rohita</i> challenged with <i>trh</i> -positive <i>V. parahaemolyticus</i> [C]: Necrosis of liver tissue and degeneration of hepatocytes (*). [D]: Blood cell infiltration (@). [E-F] Photomicrograph of kidney of <i>Labeo rohita</i> challenged with <i>trh</i> -positive <i>V. parahaemolyticus</i> [E]: Ultrastructural changes in the glomeruli. [F]: Vacuolation and necrosis of tubule (P). [G] Photomicrograph of intestine of <i>Labeo rohita</i> challenged with <i>trh</i> - positive <i>V. parahaemolyticus</i> . [G]: Intestinal mucosal epithelial cell falling off (↓) and intestinal bleeding (↓).
Figure 8.6	: SDS-PAGE profile of proteins prepared from liver tissue of control and experimentally challenge <i>Labeo rohita</i> . Red arrows indicate the differentially express protein bands.
Figure 8.7	: SDS-PAGE profile of proteins prepared from kidney tissue of control and experimentally challenge <i>Labeo rohita</i> . Red arrows indicate the differentially express protein bands.
Figure 8.8	: SDS-PAGE profile of proteins prepared from muscle tissue of control and experimentally challenge <i>Labeo rohita</i> . Red arrows indicate the differentially express protein bands.
Figure 8.9	: SDS-PAGE profile of proteins prepared from serum of control and experimentally challenge <i>Labeo rohita</i> . Red arrows indicate the differentially express protein bands.
Figure 8.10	: Inateimmune parameters in <i>Labeo rohita</i> at different time interval after experimentally challenge with <i>V. parahaemolyticus</i> . Respiratory burst activity (A); Myeloperoxidase activity (B); Lysozyme activity (C); Antiproteases activity (D); 2-Macro globulin activity (E). Bars represent mean \pm SEM (n =3 pools). Except the values of 2-Macro globulin activity and Antiproteases activity all the values are significantly different in respect to control (P < 0.05).
Figure 8.11	: Expression profiles of four different immuno genes (C3a, IL β , IL 6 and TNF α) in three different tissue (liver, kidney and muscle) during experimental challenge with <i>V. parahaemolyticus</i> .
Chapter 9	
Figure 9.1	: Position specific structural motifs identified by InterPro scan tool. Gray colour represent hit with ProDom and violet colour represent hit with Pfam database. Yellow arrow indicates beta plate and red indicates alpha helix.
Figure 9.2	: Representation of the secondary structure composition of the TRH protein. The α -helices are labeled as H and the β -strands are represented by purple arrows. The different structural motifs represented as β -turns (β), γ -turns (γ), β -hairpins (\supset), and disulfide bridge (Yellow line).

Figure 9.3	: Topology diagram of the TRH protein of <i>V. parahaemolyticus</i> was generated using the PDBSUM server. The ten β -strands are represented by pink arrows, and α -helices are represented by red cylinders.
Figure 9.4	: Phosphorylation sites predicted in the Tdh and Trh protein. The amino acids sequences of the protein are represented X-axis and the Y-axis represents the phosphorylation potential. The threshold level represented by the horizontal pink line parallel. Residues showing values above threshold level are potential phosphorylation sites.
Figure 9.5	: Representation of N-glycosylation sites predicted in the Tdh and Trh protein. The glycosylation sites within the protein are represented with yellow arrows.
Figure 9.6	: Representation of multiple sequence alignment of Tdh and Trh protein sequences of different <i>Vibrio</i> sp. and non- <i>Vibrio</i> sp. The global consensus sequence is giving at the base. Red bar at the base represents the conservedness of the sequences.
Figure 9.7	: Representation phylogenetic cladogram of the Tdh and Trh protein sequences from <i>Vibrio</i> sp. and non- <i>Vibrio</i> sp. The bootstrap percentage obtained from 1000 of samples.
Figure 9.8	: Representation of homology model of the Trh protein (A). The α -helices and β -sheets are represented in blue and red color, respectively. The coils are represented in grey color. The structures of Trh protein are superimposed with template (PDB ID:3A57) (B). The template structure showing in maroon colour.
Figure 9.9	: Ramachandran plot of the modelled Trh protein (left). The red regions within the plot symbolize the favored regions; the yellow regions symbolize the allowed regions, while the regions in pale yellow colour symbolize the generously allowed regions. Validation of the modelled structure of the Trh protein by the ProSAZ-score (right).The Z-score of all the proteins determined by X-ray crystallography in the Protein Data Bank (PDB) are represented by light blue dots while that of the structures determined by NMR are represented as dark blue dots. The Z-score of the Trh protein is represented as a large black dot.
Figure 9.10	: Root mean square deviation (RMSD) of the Trh protein over the 60 ns simulation time. Radius of gyration (Rg) of the modelled Trh protein over the 60 ns trajectory.
Figure 9.11	: Nineteen pockets have been predicted in the Trh protein. Four pockets were identified in the structure of the Trh protein, which had a large volume, and have been represented using four colours, namely, red, blue, green, and violet. The other small pockets have been shown in brown colour.