UNESCO-MIRCEN for Marine Biotechnology

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UNESCO- MIRCEN for Marine Biotechnology

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The MIRCEN at Mangalore:  
Advanced center for research in Marine Biotechnology

**Major activities:**

- Advanced research centre
- International training workshops
- Training young researchers from other countries
- UNESCO short-term Biotechnology fellowships
- Hosting guest professors
- Information resource in Marine Biotechnology and career guidance
MAJOR RESEARCH ACTIVITIES

 Development of Rapid DNA Based Methods for Detection Of Pathogens and application of these methods for Diagnosis Of Diseases and Seafood Quality Control

 Development of Vaccines and Probiotics and use in Aquaculture Systems
MAJOR RESEARCH ACTIVITIES

- Environmental Impact Of Aquaculture
- Bioremediation Of Aquatic Systems
- Bioinformatics and Its Application In Aquaculture
Research on major bacterial culture

Food pathogens:
1. *Vibrio parahaemolyticus*
   - *V. vulnificus*
   - *V. cholerae*
   - *V. harveyi*
   - *V. alginolyticus*
   - *V. anguillarum*

2. *Salmonella* Paratyphi C
   - S. Weltevreden
   - S. Oslo
   - S. Newport
   - S. Bareilly
   ...and other serotypes
Research on major bacterial culture

3. *Aeromonas hydrophila*
   - *A. caviae*
   - *A. veronii*

4. *Edwardsiella tarda*

5. *Escherichia coli*
Seafood pathogens
Human pathogenic Vibrio spp. in seafoods

*Vibrio cholerae*

*Vibrio parahaemolyticus*

*Vibrio vulnificus*
V. cholerae

- More than 200 serotypes of V. cholerae are known to occur in the Aquatic Environment.

However, only V. cholerae O1 and O139 are known to be involved in cases of Cholera.

- Non-O1, O139 V. cholerae may be occasionally involved in sporadic cases of Gastrointestinal Illness.
• Even among environmental strains of *V. cholerae* O1, non-toxigenic strains exist.

• Production of cholera toxin is encoded by *ctx* gene which is present in the genome of a filamentous bacteriophage which infects *V. cholerae*.

• Loss of bacteriophage may lead to loss of *ctx* gene and the strain may be non-toxigenic.
**V. parahaemolyticus**

- Commonly found in coastal waters all over the world
- Most of environmental strains are not pathogenic to man
- Strains involved in gastroenteritis produce a thermostable direct hemolysin (*tdh*) or a *tdh-related* hemolysin (*trh*)
**tdh** or **trh** genes can be easily detected by DNA based tests

- In our lab, we have studied the possibility of detecting **tdh**+ and **trh**+ **V. parahaemolyticus** in seafood by direct PCR and real time PCR on lysates obtained from enrichment broth.

- For detection of total **V. parahaemolyticus** PCR targeting **toxR** gene was used.

- We have also tested the possibility of detecting and enumerating **V. parahaemolyticus** in oysters by **colony hybridization** using **tdh** and **tlh** (thermolabile hemolysin) probe
V. vulnificus

- V. vulnificus is widely distributed in coastal and estuarine waters throughout the world
- Infection with V. vulnificus occurs by the ingestion of raw or undercooked shellfish, particularly oysters, or by direct entry through wounds
**Salmonella**

- A number of serotypes of *Salmonella* cause human infections.
- Traditional methods for isolation and identification take 3-4 days.
- In our lab, we have studied the possibility of detecting *Salmonella* using PCR targeting important genes encoding for pathogenicity.
Pathogenic *Escherichia coli*

Emerging as important foodborne pathogens

- Enterohemorrhagic
- Enterotoxigenic
- Enteropathogenic
- Enteroinvasive
- Enteroaggregative
Research on Fish aquaculture
**Bacterial fish pathogen**

*Aeromonas* spp.

- Ubiquitous in fresh and brackish water
- Also a pathogen of humans and lower vertebrates including amphibian, reptiles
- Most important pathogenic spp: *A. hydrophila*, *A. caviae* and *A. veronii*
- Human disease- gastroenteritis and wound infections
- Species like *A. hydrophila* clearly behaves as primary pathogen of fish
Bacterial fish pathogen

*Edwardsiella* spp.

- Species recognized: *E. tarda, E. ictaluri* and *E. hoshinae*
- *E. tarda*, major fish pathogen, leads to mass mortality in various populations and age groups of fish such as common carp, tilapia, eel, catfish, mullet, salmon, trout, flounder etc.
- Considered as an unusual human pathogen – gastrointestinal and extra-intestinal disease
Outer membrane proteins (OMPs) based vaccine for finfish (Indian Carp)

- These proteins are highly immunogenic
- OMPs are conserved
- OMPs present in the outermost layer of cell wall: epitopes exposed outside
- Present in large amounts on Gram negative bacterial surface

Collection of recombinant clones for vaccination
Bioinformatics analysis of OmpW protein of Aeromonas

Signal peptide

Predicated amino acid sequence of the OmpW protein.

Blue colour: the inner and red colour: outer region.
Underline sequences: antigenic sites

Predicted 2D structure of OmpW protein of A. hydrophila

Predicted 3D structure of OmpW protein of A. hydrophila
Multiple sequence alignment of deduced amino acid sequence of OmpW protein of different Gram negative bacteria.

Phylogenetic comparison of OmpW protein.
Comparison analysis of OmpW protein of A. hydrophila

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<th>Similarity</th>
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Multiple sequence alignment of deduced amino acid sequence of OmpW protein of A. hydrophila (n=13)
Recombinant expression and characterization of OmpW protein of *A. hydrophila*

Separation of recombinant OmpW protein on 15% SDS-PAGE

Presence of native OmpW of *Aeromonas*

Purified OmpW, 22 kDa

No expression for other Gram negative bacteria
Fish vaccination and protection efficacy of purified recombinant OMPs

- High degree of antibody production against purified recombinant OMPs in common carp

Antibody response of Aha1 and OmpW proteins vaccinated common carp

Antibody response of OmpA protein vaccinated common carp
Protection efficacy of recombinant OMPs and bacterin and crude OMPs vaccinated in rohu

Percentage survival of rohu after a challenge with *E. tarda* strains
Aqueous based vaccination in rohu

Oil (FIA) based vaccination in rohu

Antibody responses of rohu against purified OmpA protein
Shrimp aquaculture:
Indian Tiger Shrimp
(P. monodon)
Research on the shrimp viral diseases

White spot Syndrome Virus (WSSV)
Monodon Baculovirus (MBV)
Hepatopancreatic Parvovirus (HPV)
Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)
Size variations observed in 50 days old infected *Penaeus monodon*
Vibrios:  *Vibrio harveyi*

- Inhabitant of tropical marine environment
- One of 6 luminous *Vibrio* spp
- Highly pathogenic to aquatic fauna - vibriosis
- Considered as opportunistic pathogen
Approaches taken for shrimp disease management

**PATHOGEN**
- Rapid detection
- Suppression using probiotics

**ENVIRONMENT**
- Bioremediation

**HOST**
- Improving disease resistance
- Immunostimulants
Phage therapy

Collection of *V. harveyi* phage

TEM of *V. harveyi* Phage

Magnification: 85,000 X
Head dia: 40-45nm,
Tail dia: 7nm
Tail length: 160nm

Electron micrograph of negatively stained *V. harveyi* Phage
UNESCO-MIRCEN Activities

- UNESCO short-term Biotechnology fellowships and International training workshops
Visits by Guest researchers
UNESCO Biotechnology Fellowship

Ms. Anna Godhe
University of Goteborg, Sweden

Mr. Dante Mateo
Universidad National Federico Villarreal Lima, Peru

Ms. Yuedan Li
Institute of Microbiology
Chinese Academy of Sciences, Beijing

Dr. Min Wang
Department of Marine Biology, Ocean University of Qingdao, China

Prof. Huai Xu
Ocean University of Qingdao, China

Dr. Ann Sofi Rehnstam-Holm
Department of Medical Bacteriology, University of Goteborg, Sweden
Visit By Dignitaries

Dr. Rita R. Colwell, Director NSF USA
Dr. David James, Fisheries Officer, FAO, ROME
Dr. John Sumner, FAO consultant, AUSTRALIA
Dr. Mike Phillips, Environment specialist NACA, THAILAND
Dr. Pornlerd Chanratchakool, AAHRI, THAILAND
Dr. C. Lavilla –Pitogo, SEAFDEC, PHILIPPINES
Dr. Werner Goebel, University Wurzburg, GERMANY
Dr. Richard Fuchs, IFS Scientific secretary, SWEDEN
Dr. Allen Reilly, ODNRI, Chatham U.K.
Dr. Sandra Adams & Dr. Kim Thompson, Institute of Aquaculture, STIRLING, U.K.
Dr. Huai Shu Xu, Ocean Univ. of Qingdao, PR CHINA
Dr. Peter Ben Embarek, WHO, GENEVA
UNESCO - MIRCEN
International Workshops

1996: ODA/UNESCO MIRCEN
International workshop:
Molecular techniques for
detection of pathogens
associated with seafoods

1999: FAO/UNESCO MIRCEN
International Workshop:
Risk assessment for
pathogens associated
with seafoods
2000: FAO/UNESCO MIRCEN
International Workshop: Biotechnological tools in pathogen detection and fish/shellfish health management

2003: UNESCO International Workshop: Water and waste water microbiology
UNESCO MIRCEN
International Workshops

2006: FAO/ UNESCO MIRCEN
International Workshop: Molecular techniques in aquaculture and seafood safety

2010: FAO/ UNESCO MIRCEN
International Workshop: Biosecurity measures for control of *Salmonella* contamination in sustainable aquaculture
Thank you for your kind attention