

❖ **Project title: “Immunoprophylaxis at early life cycle stages of the Asian seabass (*Lates calcarifer*) with emphasis on streptococci infections”**

❖ **Objectives:** to develop strategies for immune prevention at early life cycle stages of farmed Asian sea bass through detailed studies of virulence mechanisms of the pathogenic bacteria *Streptococcus iniae*.

❖ **Contents:** The research activity is divided into 5 work packages:

- Study the ontological features of Asian sea bass with emphasis on the development of the thymus and the early occurrence of B lymphocyte in lymphoid organs.
- Collection of isolates of *Streptococcus iniae* from clinical outbreaks of disease in sea bass.
- A detailed characterization of the collected bacterial isolates. This will include sequence data of the bacterial 16S rDNA and virulence characteristics in experimental challenge models. Avirulent isolates will be used in challenge and vaccination studies.
- Studies of antigen uptake following exposure to inactivated and attenuated/avirulent strains of bacteria using *in situ* and *in vitro* methods for studies of and detection of uptake.
- Studies of obtained immune protection in early life cycle stages of fry of Asian sea bass using inactivated and attenuated/avirulent bacterial isolates, with focus on safety and efficacy.

❖ **Implementation group:**

1. Prof. Dr. Heidrun Inger Wergeland – University of Bergen– Technical Advisor
2. Dr. Nguyen Huu Dung, team leader
3. Mr. Tran Vi Hich, PhD candidate
4. Mrs. Vu Đang Ha Quyen, Master student
5. Mrs. Nguyen Thi Thoa, Master student
6. Miss Dang Thi Sao Mai, Undergraduate student
7. Miss Nguyen Thi Mai Anh, Undergraduate student

❖ **Results:**

- Study the ontological features of Asian sea bass with emphasis on the development of the thymus and the early occurrence of B lymphocyte in lymphoid organs: sea bass larvae were collected from 5 batches of spawning at 6-hour interval from the time of hatching till 25 days post-hatching. The larvae were fixed with buffered formalin, serially sectioned and stained with Haematoxylin and Eosin to observe the development of the fish immune system. The fish thymus fully develops at day 14 post-hatch. The result showed that immuno-prophylaxis can only apply to sea bass from day 14 post-hatch. Related publications/ disseminations (*See appendix 7*).
- Eight strains of *Streptococcus iniae* were collected from clinical outbreaks of disease in sea bass cultured in various farms in Khanh Hoa province. All the isolates were

classified as *Streptococcus iniae* based on morphological, bio-chemical and genetical (16S rDNA sequence) characteristics. The isolates' 16S rDNA sequence showed 99% homology to the type-strain of *Streptococcus iniae* of the American Tissue Culture Collection (ATCC-29178). Virulence characteristics of the bacterial isolates were determined through experimental challenges. The transmission experiments showed that all the isolates are highly pathogenic to sea bass with LD₅₀ from 10^{4.8} - 10^{5.9} CFU/ml. Avirulent isolate was not found in this study. Related publications/ dissemination (See appendix 7).

- Studies of antigen uptake following exposure to inactivated and attenuated/avirulent strains of bacteria using *in situ* and *in vitro* methods for studies of and detection of uptake: Natural immune response of sea bass fingerlings to the *Streptococcus iniae* isolates were studied. The results showed that there is no statistical difference between the fish immunized with the isolates' bacterin and the control group in term of natural cellular immunity, i.e macrophage index (2.1 in immunized fish vs 1.8 in control fish), and macrophage activity (52% vs 44% respectively). However, humoral natural immune response was significantly different between the immunized and non-immunized fish with serum lysozymes concentrations of 16µg/ml and 10µg/ml respectively. Related publications/ dissemination (See appendix 7).
- Studies of obtained immune protection in early life cycle stages of fry of Asian sea bass using inactivated and attenuated/avirulent bacterial isolates, with focus on safety and efficacy: Sea bass fingerlings were immunized with formalin-killed bacterin and challenged with the *Streptococcus iniae* isolates at 3 weeks post-immunization. The result showed that the inactivated vaccine effectively protected the experimental fish from Streptococcosis with Relative Percent Survival (RPS) as high as 92%. Related publications/ dissemination (See appendix 7).

❖ **Papers published:** 1 paper published in an international journal

3 papers presented at national conferences

3 papers presented at international conferences

3 papers published in national journals

3 papers accepted by national fisheries magazines

❖ **Training:**

- PhD student: Mr. Tran Vi Hich. Thesis title: "Study on the immune response of the barramundi (*Lates calcarifer* Bloch, 1790) larvae against *Streptococcus iniae*". The thesis was submitted and pending for defence at the evaluation committee.
- Master students:
 1. Vu Dang Ha Quyen. Completed at University of Bergen, Norway. Thesis title: "Analyses of the fish pathogenic bacteria *Streptococcus iniae*". (abstract copy attached)
 2. Nguyen Thi Thoa. Completed at Nha Trang University. Thesis title: "Virulence and protein profiles of the *Vibrio alginolyticus* strains isolated

from cage-cultured barramundi (*Lates calcarifer*) in Khanh Hoa province infected with ulcerative syndrome” .

❖ **Sustainability:** Immunoprophylaxis for fish disease prevention is an important issue for sustainable aquaculture development in Vietnam as well as Southeast Asian countries. Aquaculture sectors, particularly the sea bass farms in Vietnam, are interested in the implementation progress and achievement of the current study. It is expected the success of the study will help to develop an effective prevention strategy for sea bass farming against Streptococcosis and thus enabling the sustainability of the research activity in the specific field at Nha Trang University in future.

❖ **Technology transfer**

- The 8 strains of *Streptococcus iniae* collected through the current study were transferred to Pharmaq Vietnam Co. Ltd., a Norwegian vaccine producer, with permission of the Rector of Nha Trang University and The Royal Norwegian Embassy to Vietnam in Hanoi, in order to be used as antigen source for developing commercial vaccines against Streptococcosis in marine finfish farming in tropical areas.
- The company also established collaboration with Nha Trang University in this field of study. Under the written permission of The Rector of Nha Trang University, Dr. Nguyen Huu Dung, the research team leader and Director of NTU's Center for Aquatic Animal Health and Breeding Studies (CAAHBS), negotiated and signed the legal documents with Pharmaq Vietnam Co.,Ltd. The collaboration was initiated from the second quarter of 2012.
- Australis Vietnam Co. Ltd, an FDI sea bass farming company based in Khanh Hoa province, has express its interest in the achievement of the current study and the possible collaboration between Pharmaq Vietnam Co. Ltd, and NTU as the company has been facing problem of streptococcosis in the company cultured sea bass. Australis Vietnam Co. Ltd. is willing to support the experimental application of the inactivated vaccine to the sea bass cultured in the company sea cages in Khanh Hoa province.