# CHARACTERIZATION OF MICROORGANISMS IN BIOFLOC FORMATION OF PACIFIC WHITE LEG SHRIMP (*Penaeus vannamei*) IN GROW-OUT POND

NURARINA AYUNI BINTI GHAZALI

MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU
2016

# CHARACTERIZATION OF MICROORGANISMS IN BIOFLOC FORMATION OF PACIFIC WHITE LEG SHRIMP (*Penaeus vannamei*) IN GROW-OUT POND

NURARINA AYUNI BINTI GHAZALI

Thesis Submitted in Fulfillment of the Requirement for the
Degree of Master of Science in the
Institute of Tropical Aquaculture,
Universiti Malaysia Terengganu

May 2016

This one is for you, ***Abah*** and ***Ummi*** :)
For all the supports, kindness and countless prayers, may Allah grants you the highest place in Jannah.

*One. Step. Closer.*

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science

**CHARACTERIZATION OF MICROORGANISMS IN BIOFLOC FORMATION OF PACIFIC WHITE LEG SHRIMP (*Penaeus* vannamei) IN GROW-OUT POND**

**NURARINA AYUNI BINTI GHAZALI**

**May 2016**

**Supervisor** : **Nor Azman Bin Kasan, Ph.D.**

**Co-Supervisor** : **Professor Nakisah Mat Amin, Ph.D.**
**Associate Professor Noraznawati Ismail, Ph.D.**

**Institute** : **Institute of Tropical Aquaculture**

An environmental friendly technology known as Biofloc Techology (BFT) has been developed to reduce environmental damages and optimise production of aquaculture industry. In BFT, microbial communities in biofloc are responsible in eliminating water exchange and producing microbial proteins that can be used as supplementary feed for shrimp culture. Knowledge on microbial composition of biofloc is important for successful design and application of BFT. However, there are no intensive study on the microbial composition of biofloc. Therefore, this study was conducted to identify the microbial communities of biofloc in shrimp pond including bacteria, fungi and microalgae through morphological and molecular identification. In addition, phylogenetic relationship between all isolated microorganisms was evaluated through phylogenetic tree analysis. Water quality of shrimp pond throughout biofloc formation was also analysed. Water and biofloc samples were collected at ten days interval starting from Before Water Treatment (BWT) to 70 Days of Culture (DOC). In this study, biofloc was observed to be formed at DOC 30 up to DOC 70.

Out of 232 bacterial isolates, nine species of bacteria (*Halomonas venusta*, *H. aquamarina*, *Vibrio parahaemolyticus*, *V. alginolyticus*, *Bacillus infantis*, *B. cereus*, *B. safensis*, *Nitratireductor aquimarinus* and *Pseudoalteromonas* sp.) were identified. For fungi, out of 127 isolates, eight species of fungi (*Aspergillus versicolor*, *A. niger*, *A. tamarii*, *A. flavipes*, *A. aculeatus*, *Penicillium citrinum*, *P. griseofulvum* and *Pestalotiopsis microspora*) were identified. From 102 isolates of microalgae, three species of microalgae (*Chlorella vulgaris*, *Nannochloropsis oceanica* and *Ulothrix* sp.) were identified. High to moderate bootstrap value in phylogenetic analysis of all microorganisms indicated that there is a phylogenetic relationship between the microorganisms such as sharing common ancestor and taxa group. Presence of biofloc in shrimp pond has successfully maintained water quality of shrimp pond as well as reducing nutrient concentration to a safe level for shrimp culture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

# PENCIRIAN MIKROORGANISMA DALAM PEMBENTUKAN BIOGUMPAL DALAM KOLAM TERNAKAN TUMBESARAN UDANG PUTIH (*Penaeus vannamei)*

**NURARINA AYUNI BINTI GHAZALI**

**Mei 2016**

**Penyelia** : **Nor Azman Bin Kasan, Ph.D.**

**Penyelia bersama** : **Profesor Nakisah Mat Amin, Ph.D.**
**Profesor Madya Noraznawati Ismail, Ph.D.**

**Institut** : **Institut Akuakultur Tropika**

Satu teknologi mesra alam yang dikenali sebagai Teknologi Biogumpal (BFT) telah dibangunkan untuk mengurangkan kerosakan alam sekitar dan mengoptimumkan pengeluaran industri akuakultur. Di dalam BFT, komuniti mikrob di dalam biogumpal bertanggungjawab dalam menyingkirkan pertukaran air dan menghasilkan protein mikrob yang boleh digunakan sebagai makanan tambahan untuk kultur udang. Pengetahuan tentang komposisi mikrobial biogumpal penting untuk kejayaan reka bentuk dan aplikasi BFT. Tetapi, tiada kajian intensif yang telah dijalankan ke atas komposisi mikrob biogumpal. Oleh itu, kajian ini dijalankan untuk mengenal pasti komuniti mikrobial di dalam biogumpal di dalam kolam udang seperti bakteria, fungus dan mikroalga melalui identifikasi morfologi dan "molecular". Sebagai tambahan, hubungan filogenetik di antara semua mikroorganisma yang dipencilkan dinilai melalui analisis pokok filogenetik. Kualiti air kolam udang sepanjang pembentukan biogumpal juga dinilai. Sampel air dan biogumpal telah dikumpulkan pada selang sepuluh hari bermula daripada Sebelum

Rawatan Air (BWT) sehingga 70 Hari Kultur (DOC). Dalam kajian ini, biogumpal dilihat mula terbentuk pada DOC 30 sehingga DOC 70.

Daripada 232 "isolates" bakteria, sembilan spesis bakteria (*Halomonas venusta*, *H. aquamarina*, *Vibrio parahaemolyticus*, *V. alginolyticus*, *Bacillus infantis*, *B. cereus*, *B. safensis*, *Nitratireductor aquimarinus* dan *Pseudoalteromonas* sp.) telah dikenal pasti. Untuk fungi, daripada 127 "isolates", lapan spesis fungus (*Aspergillus versicolor*, *A. niger*, *A. tamarii*, *A. flavipes*, *A. aculeatus*, *Penicillium citrinum*, *P. griseofulvum* dan *Pestalotiopsis microspora*) telah dikenal pasti. Daripada 102 "isolates" mikroalga, tiga spesis mikroalga (*Chlorella vulgaris*, *Nannochloropsis oceanica* dan *Ulothrix* sp.) telah dikenal pasti. Nilai "bootstrap" yang tinggi hingga sederhana di dalam analisis filogenetik bagi semua mikroorganisma menandakan bahawa terdapat kewujudan hubungan filogenetik diantara mikroorganisma tersebut seperti berkongsi keturunan yang sama dan kumpulan taxa. Kehadiran biogumpal di dalam kolam udang telah berjaya mengekalkan kualiti air kolam udang dan juga mengurangkan kepekatan nutrien kepada tahap yang selamat untuk kultur udang.