

**DIVERSITY OF BACTERIAL COMMUNITY  
ASSOCIATED WITH MARINE ASCIDIANS  
FROM BIDONG ISLAND, TERENGGANU**

**NUR AZNA SAARI**

**MASTER OF SCIENCE**

**2014**

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**Thesis Submitted in Fulfillment of the Requirements  
for the Degree of Master of Science in the School of  
Fisheries and Aquaculture Sciences  
Universiti Malaysia Terengganu**

**May 2014**



Abstract of thesis presented to the Senate of University Malaysia Terengganu in fulfilment for the degree of Master of Science

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**School : School of Fisheries and Aquaculture Sciences**

Microbial diversity has been well documented for many marine invertebrate taxa. However, little attention has been given to the examination of microbial–tunicates associations. In the present study, the microbial diversity of marine tunicates was assessed by using both culture-dependent and culture-independent method. Two species of tunicates from *Clavelina* sp. and *Didemnum* sp., have been collected from Bidong Island, Terengganu, during pre-monsoon (October) and post-monsoon seasons (May). Bacteria were isolated by serial dilutions and characterized based on colony and cell morphology. All bacteria isolates were identified based on 16S rDNA sequences. In addition, metagenomes were directly extracted from tunicate. Amplification of full length 16S rRNA gene was then carried out. The amplicon were used as template to amplify the V3 region before subjected to Illumina 16S metagenomic sequencing. The high numbers of sequences were trimmed and assembled. Then, all sequences were classified based on RDP classifier. A total of 89 bacteria were isolated from Blue bell tunicates, *Clavelina* sp. and Green barrel tunicates, *Didemnum* sp. where 46 bacteria isolated from *Clavelina* sp. and 43 bacteria isolated in *Didemnum* sp., Among this, most of bacteria isolates from *Clavelina* sp. and *Didemnum* sp. showed phylum of  $\gamma$ -Proteobacteria were the

dominant class during pre-monsoon and post-monsoon season. The analysis of the 16S rRNA metagenomic revealed that the phylum of *Proteobacteria* associated with both tunicates was dominated with  $\alpha$ -*Proteobacteria* as the major component in *Clavelina* sp. and *Didemnum* sp. The result revealed the presence of other taxa such as *Bacteroidetes*, *Fusobacteria*, *Acidobacteria*, *Nitrospira*, *Tenericutes*, *Planctomycetes*, *Actinobacteria*, TM7 were found in this study. The results indicated that *Didemnum* sp. hosts was more diverse microbiota when compared to *Clavelina* sp. during pre-monsoon season. This is the first study where in seasonal changes in bacterial diversity associated with the marine tunicates has been attempted in the Malaysian water and the present study can provides new data into the bacteria diversity associated with tunicates isolated from Bidong Island.

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

**KEPELBAGAIAN KOMUNITI BAKTERIA YANG BERSEKUTU DENGAN  
SPESIES ASCIDIAN DARI PULAU BIDONG, TERENGGANU**

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**Mei 2014**

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Kepelbagaian komuniti mikrob dalam haiwan tidak bertulang belakang telah banyak didokumentasikan. Walau bagaimanapun, hanya sedikit perhatian yang diberikan untuk kajian tentang hubungan antara tunikat dan bacteria yang bersekutu. Dalam kajian ini, kepelbagaian marin mikrob tunikat dikaji dengan menggunakan kedua-dua kaedah pengkulturan dan kaedah tanpa pengkulturan. Dua spesies tunicates dari *Clavelina* sp. dan *Didemnum* sp., telah diperolehi dari Pulau Bidong, Terengganu, semasa musim pra-monsoon (Oktober) dan musim pasca monsoon (Mei). Bacteria telah dipencilkan melalui kaedah pencairan bersiri dan dikelas berdasarkan bentuk koloni dan morfologi sel. Kemudian, metagenom diekstrak secara langsung daripada tunikat. Amplifikasi 16S rRNA kemudiannya dijalankan. Amplikon yang terjana digunakan sebagai templat untuk amplifikasi kawasan V3 sebelum penjujukan 16S metagenomik dilakukan. Jutaan jujukan yang terjana kemudian disatukan. Selepas itu, semua jujukan dikelaskan menggunakan RDP Classifier. Sebanyak 89 bacteria telah dipencilkan daripada tunikat, *Clavelina* sp. dan *Didemnum* sp. di mana, 46 jumlah bacteria dipencilkan daripada *Clavelina* sp. dan 43 jumlah bacteria dipencilkan dalam *Didemnum* sp., Dalam jumlah ini, bacteria

yang dipencil daripada *Clavelina* sp. dan *Didemnum* sp. telah menunjukkan kelas dari  $\gamma$ -*Proteobacteria* adalah paling dominan semasa pra-monsoon dan pasca-musim monsoon. Analisis daripada 16S rRNA Metagenomik telah mendedahkan hubungan filum proteobakteria simbiosis dengan kedua-dua tunicates iaitu didominasi oleh kelas dari  $\alpha$ - *Proteobakteria* sebagai komponen utama dalam *Clavelina* sp. dan *Didemnum* sp. Hasil kajian menunjukkan kehadiran taksa lain seperti *Bacteroidetes*, *Fusobakteria*, *Acidobakteria*, *Nitrospira*, *Tenericutes*, *Planctomycetes*, *Actinobakteria* TM7 ditemui dalam kajian ini. Kajian ini, mampu menjadi perintis kepada kepelbagaian bakteria yang bersekutu dengan tunikat yang dijumpai di Pulau Bidong, dan mampu meningkatkan maklumat semasa hubungan antara tunikat dan bakteria marin.