

**IDENTIFICATION OF MARINE BACTERIA COLLECTED FROM
HEALTHY AND BLEACHED *ACROPORA* SP.
IN PASIR AKAR, PULAU REDANG**

NOOR SURINA OTHMAN

**FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU**

2008

1100061856

Universiti Malaysia Terengganu

In 6431

LP 35 FMSM 1 2008



1100061856

Identification of marine bacteria collected from healthy and bleached *Acropora* sp. in Pasir Akar, Pulau Redang / Noor Surina Othman.



PERPUSTAKAAN SULTANAH NUR ZAHIRAH
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

1100061856

Lihat sebelah



**IDENTIFICATION OF MARINE BACTERIA COLLECTED
FROM HEALTHY AND BLEACHED *ACROPORA* SP.
IN PASIR AKAR, PULAU REDANG**

By

Noor Surina Binti Othman

Research Report submitted in partial fulfillment of
the requirements for the degree of
Bachelor of Science (Marine Biology)

Department of Marine Science
Faculty of Maritime Studies and Marine Science
UNIVERSITI MALAYSIA TERENGGANU
2008

This project should be cited as:

Noor Surina, O., 2008. Identification of marine bacteria collected from healthy and bleached *Acropora* sp. in Pasir Akar, Pulau Redang. Undergraduate thesis, Bachelor of Science (Marine Biology), Faculty of Maritime Studies and Marine Science, Universiti Malaysia Terengganu, Terengganu. 57p.

No part of this project report may be reproduced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied from public or private use, without written permission from the author and the supervisor(s) of the project.



JABATAN SAINS MARIN
FAKULTI PENGAJIAN MARITIM DAN SAINS MARIN
UNIVERSITI MALAYSIA TERENGGANU

PENGAKUAN DAN PENGESAHAN LAPORAN
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

Identification of Marine Bacteria Collected from Healthy and Bleached *Acropora* sp. in Pasir Akar, Pulau Redang oleh Noor Surina Binti Othman, No.Matrik **UK 11440** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Marin sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Biologi Marin), Fakulti Pengajian Maritim dan Sains Marin, Universiti Malaysia Terengganu.

Disahkan oleh:

Penyelia Utama

Nama: **YM TENGKU FARAKAMILIA TG MOHD KAMIL**

Pensyarah

Jabatan Sains Marin

Fakulti Pengajian Maritim dan Sains Marin
Universiti Malaysia Terengganu
(UMT)

Tarikh: **4 MEI 2008**

Penyelia Kedua

DR. MARIAM TAIB

Pensyarah

Jabatan Sains Biologi

Cop Rasmi: **Fakulti Sains dan Teknologi**
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Tarikh: **4/5/08**

Ketua Jabatan Sains Marin

Nama: **DR. RAZAK ZAKARIYA**

Ketua Jabatan Sains Marin

Cop Rasmi: **Fakulti Pengajian Maritim dan Sains Marin**
Universiti Malaysia Terengganu
(UMT)

Tarikh: **12/5/08**

ACKNOWLEDGEMENTS

First of all, I would like to express my heartfelt thanks to my supervisor, Y.M Puan Tengku Fara Kamilia binti Tengku Kamil for her willingness to accept me as her student and guided me throughout almost one year to accomplish this project, and also for financial support to conduct this study. Thanks to Dr. Mariam Taib also for her aids in laboratory works to ensure that this project could be carried on properly.

Special thanks to my beloved parents, En. Othman Salleh and Pn. Maimunah bti Wahid for their unlimited support and understand me as a student, must be willing to sacrifice time with the family. For my parents, I will never ever let you down.

Lots of thank also to the laboratory assistants, En. Matzan, En. Syed, En. Jalal, Pn. Kartini and the rest of the assistants for helping me to deal with the tools and machines that were needed during sampling and also during laboratory works.

Last but not least, thanks to all my friends for assisting and supporting me and each other to make sure that this project could be accomplished on time. I will always remember the moments we spent together.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	x
LIST OF APPENDICES	xi
ABSTRACT	xii
ABSTRAK	xiv
CHAPTER 1: INTRODUCTION	
1.1 Introduction	1
1.2 Significance of Study	2
1.3 Objectives	3
CHAPTER 2: LITERATURE REVIEW	
2.1 Coral Reef Ecology	4
2.2 <i>Acropora</i> Corals	5
2.3 Pulau Redang Reef Status	6
2.4 Health of Corals	7
2.4.1 Healthy corals	7
2.4.2 Unhealthy corals	8

2.5	Marine Bacteria	11
2.5.1	Coral bacteria	13
2.5.2	Coral bacteria association	14

CHAPTER 3: METHODOLOGY

3.1	Sampling	17
3.2	Isolation of Bacteria	18
3.3	Morphological Tests	18
3.3.1	Gram staining	18
3.3.2	Acid fast stain (Ziehl-Neelsen method)	19
3.3.3	Endospore stain	19
3.4	Basic Phenotypic Characteristics Tests	20
3.4.1	Catalase test	20
3.4.2	Oxidase test	20
3.4.3	Indole production	20
3.4.4	Methyl red test	21
3.4.5	Voges-Proskauer test	21
3.4.6	Triple Sugar Ion (TSI) agar test	22
3.5	Bacteria identification	22

CHAPTER 4: RESULTS

4.1	Sampling	23
4.2	Bacteria Isolation	23

4.3	Bacteria Identification	24
-----	-------------------------	----

CHAPTER 5: DISCUSSION

5.1	Coral Bleaching	28
5.2	Coral Tissue Bacteria	28
5.3	Comparison on Availability of <i>Acropora</i> sp. Bacteria	29
5.4	Distribution of Identified Bacteria	30
5.5	Groups of Identified Bacteria	33
5.6	Roles of Coral Bacteria	34

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS		36
--	--	----

REFERENCES	38
-------------------	----

APPENDICES	44
-------------------	----

CURRICULUM VITAE	57
-------------------------	----

LIST OF TABLES

	Page
4.1 Sampling location and sea water parameters	23
4.2 List of identified bacteria from healthy and bleached <i>A.formosa</i> and <i>A.nobilis</i>	25
5.1 Habitats of identified bacteria from <i>A.formosa</i> and <i>A.nobilis</i>	31

LIST OF FIGURES

	Page
3.1 Overview of methodology according to culture-based methods	16
3.2 Map of sampling site in Pasir Akar (Source: Google Earth)	17
4.1 Proportions of identified bacteria from <i>A. formosa</i> and <i>A. nobilis</i> according to its group	26
4.2 Number of Gram-positive (+) and Gram-negative (-) bacteria identified from <i>A. formosa</i> and <i>A. nobilis</i>	26
4.3 Percentage for proportions of Gram-positive (+) and Gram-negative (-) bacteria identified from <i>A. formosa</i> and <i>A. nobilis</i>	27

LIST OF ABBREVIATIONS

α	-	alpha
β	-	beta
γ	-	gamma
μl	-	microlitre
$^{\circ}\text{C}$	-	degree Celcius
s	-	second
min	-	minute
m	-	meter
sp.	-	species
ml	-	mililitre
mm	-	millimeter
km	-	kilometer
cm	-	centimeter
ppt	-	parts per thousand

LIST OF APPENDICES

	Page
1 Gram stained picture of bacteria from healthy <i>A.formosa</i> under light compound microscope (1000x) (a) <i>Paracoccus</i> sp. (b) <i>Alcaligenes</i> sp. (c) <i>Azotobacter</i> sp. (d) <i>Oceanospirillum</i> sp.	44
2 Gram stained picture of bacteria from bleach <i>A.formosa</i> under light compound microscope (1000x) (a) <i>Mesophilobacter marinus</i> (b) <i>Arthrobacter</i> sp. (c) <i>Microbacterium</i> sp. (d) <i>Brucella</i> sp. (e) <i>Micrococcus</i> sp. (f) <i>Methylophaga</i> sp. (g) <i>Pseudomonas</i> sp. (h) <i>Brochothrix</i> sp. (i) <i>Caryophanon</i> sp.	45
3 Gram stained picture of bacteria from healthy <i>A.nobilis</i> under light compound microscope (1000x) (a) <i>Bacillus</i> sp. (b) <i>Aeromicrobium</i> sp. (c) <i>Syntrophospora</i> sp. (d) <i>Moraxella</i> sp. (e) <i>Salinicoccus</i> sp. (e) <i>Pseudomonas</i> sp.	47
4 Gram stained picture of bacteria from bleach <i>A.nobilis</i> under light compound microscope (1000x) (a) <i>Azomonas</i> sp. (b) <i>Vagococcus</i> sp. (c) <i>Acetobacterium</i> sp. (d) <i>Brochothrix</i> sp.	48
5 Differential characteristics of bacteria from healthy <i>A.formosa</i>	49
6 Differential characteristics of bacteria from bleached <i>A.formosa</i>	50
7 Differential characteristics of bacteria from healthy <i>A.nobilis</i>	52
8 Differential characteristics of bacteria from bleached <i>A.nobilis</i>	54
9 Groups of identified bacteria from <i>A.formosa</i> and <i>A.nobilis</i>	55
10 <i>A.formosa</i> and <i>A.nobilis</i>	56

ABSTRACT

This study was conducted in Pasir Akar, Pulau Redang. The objectives of this study are to isolate and identify the types of bacteria present in healthy and bleached tissue of *Acropora* sp. Fragments of healthy and bleached *Acropora formosa* and *A.nobilis* corals were taken at the depth of 10 m with the aids of scuba gear and brought back to the laboratory for further analysis and identification. Culture-based methods were applied to isolate the bacteria from the coral tissues and identified by referring to Bergey's Manual of Determinative Bacteriology. Overall, there were 23 types of bacteria that had been successfully isolated and identified. *A.formosa* was found to harbor 13 types of bacteria; four from healthy coral colony and nine from bleached coral colony. Meanwhile for *A.nobilis*, 10 types of bacteria were to associate with its tissue; six from healthy colony and four from bleached colony. Some examples of bacteria that had been successfully identified include *Alcaligenes* sp., *Azotobacter* sp., and *Micrococcus* sp. However, there is overlapping of bacteria existence where *Pseudomonas* sp. and *Brochothrix* sp. are inhabit both of *A.formosa* and *A.nobilis*. All of the bacteria were known to inhabit marine environment except *Acetobacterium* sp. and *Caryophanon* sp. About 62% of the bacteria are Gram-positive and 38% are Gram-negative. The most dominant groups are affiliated to γ -proteobacteria class and Firmicutes which represent 33% of the total bacteria isolated respectively, and then followed by Actinobacteria (19%), α -proteobacteria (10%) and lastly β -proteobacteria (5%). Some of the bacteria may play important roles in the interaction of coral-bacteria such as nitrogen fixer and secrete antibiotic substances to

protect their host, which were the corals itself. However, the overall roles played by these bacterial communities are still relatively unknown.

PENGECAMAN BAKTERIA MARIN DARIPADA *ACROPORA* SP. YANG SIHAT DAN LUNTUR DI PASIR AKAR, PULAU REDANG

ABSTRAK

Kajian ini telah dijalankan di Pasir Akar, Pulau Redang. Objektif kajian ini adalah untuk mengasing dan mengenal pasti jenis bakteria daripada *Acropora* sp. yang sihat dan luntur. Sampel daripada karang *Acropora formosa* dan *A.nobilis* yang sihat dan luntur telah diambil pada kedalaman 10 m dengan menggunakan peralatan scuba dan dibawa ke makmal untuk analisis dan pengecaman. Kaedah berdasarkan kultur telah diaplikasikan bagi mengasingkan bakteria daripada tisu karang dan pengecaman telah dilakukan dengan merujuk kepada Bergey's Manual of Determinative Bacteriology. Keseluruhannya, 23 jenis bakteria telah berjaya diasingkan dan dikenalpasti. *A.formosa* diketahui mempunyai sebanyak 13 jenis bakteria; empat daripada koloni karang yang sihat dan sembilan daripada koloni karang yang luntur. Sementara itu, *A.nobilis* pula telah dikenal pasti mempunyai 10 jenis bakteria, yang mana enam daripadanya datang daripada sampel karang yang sihat dan empat jenis daripada sampel karang yang luntur. Contoh-contoh bakteria yang telah berjaya dikenalpasti adalah *Alcaligenes* sp., *Azotobacter* sp. dan *Micrococcus* sp. Walau bagaimanapun, terdapat pertindihan berlaku di mana *Pseudomonas* sp. dan *Brochothrix* sp. dijumpai mendiami kedua-dua *A.formosa* dan *A.nobilis* yang sihat dan luntur. Kesemua bakteria yang dijumpai diketahui hidup di persekitaran marin kecuali *Acetobacterium* sp. dan *Caryophanon* sp. Kira-kira 62% daripada bakteria yang dikenalpasti adalah Gram-positif manakala 38% lagi adalah Gram-negatif. Kumpulan bakteria yang dominan adalah kelas γ -proteobakteria dan

Firmicutes yang masing-masing mewakili 33% daripada jumlah bakteria yang dikenalpasti, dan diikuti oleh Actinobacteria (19%), α -proteobakteria (10%) dan β -proteobakteria (5%). Sebahagian daripada bakteria karang ini memainkan peranan yang penting dalam interaksi karang-bakteria seperti pengikat nitrogen dan juga menghasilkan bahan antibiotik bagi melindungi karang itu sendiri yang menjadi hosnya. Walaubagaimanapun, keseluruhan fungsi dan peranan yang dimainkan oleh komuniti bakteria ini terhadap hosnya masih belum diketahui.