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ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF CORAL MUCUS ASSOCIATED BACTERIA (ACROPORA CERVICORNIS) FROM BIDONG ISLAND, TERENGGANU MALAYSIA

By

Murugan Kalimutho

Research Report submitted in partial fulfillment of
the requirements for the degree of
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Department of Biological Sciences
Faculty of Science and Technology
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
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Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk: **ISOLATION CHARACTERIZATION AND IDENTIFICATION OF CORAL MUCUS ASSOCIATED BACTERIA (*Acropora cervicornis*) FROM BIDONG ISLAND, TERENGGANU MALAYSIA** oleh **MURUGAN KALIMUTHO**, no. matrik: **UK 8813** telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah **SARJANA MUDA SAINS (SAINS BIOLOGI)**, Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

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Whatever that has happened, was for good

Whatever that is happening, is also for good

Maybe whatever that happen in future, ought to also be for good

What have you lost, that is yours that you are weeping?

What have you brought, for you to lose it?

What have you created for it to be wasted?

Whatever that you took, you have taken it from here (me)

Whatever that you gave, you have given it to this Universe (me)

Whatever that is yours today, will be somebody else's in future

This is universal principle and the essence of my creation

-Bhagavan Sri Krishna-

Smile

A Smile costs nothing, but gives much. It enriches those who receive, without making poorer those who give. It takes a moment, but the memory of it sometimes lasts forever. None is so rich or might that he can get along without it; none is so poor but that he can be made rich by it. A smile creates happiness in the home, fosters good will in business, is the countersign of the weary, cheer to the discouraged, sunshine to the sad, and it is nature's best antidote for trouble. Yet it cannot be brought, begged, borrowed, or stolen, for it is something that no value to anyone until it is given away. Some people are tired to give you a smile. Give them one of yours, as none needs a smile as much as he who has no more to give

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LIST OF ABBREVIATIONS

Bp	base pair
C	cytosine
DNA	deoxyriribonucleic
dNTP	2'-deoxynucleoside-5';triphosphate(s)
G	Guanine
NTSYS	Numerical Taxonomy and Multivariate Analysis System
OD	optical Density
PCR	polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
SAHN	Seguential, Aggomerative,Hierarchical and Nested Clustering
TE	10mMTris-Cl, 1mM EDTA
UPGMA	Unweighted Paired-Group Method of Aritmetic
VDS	Video Documentation System
w/v	Weight/ Volume
µM	Micrometer
E'	East
N'	North
F	Fermentative
H_2S	Hydrogen Sulfate
NaCl	Sodium Chloride
m	Meter
min	Minute
Sec	Second
MR	Methyl Red

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ABSTRACT

Isolation. characterization and identification of bacteria from mucus of *Acropora cervicornis* collected from Bidong Island Terengganu were achieved by using biochemical test and molecular technique. The bacteria were isolated using specific microbiology method and cultured in Sucrose Sea Water agar (SSW). The morphology and physiological characteristics were tested in various media such as MacConkey agar. Pseudomonas agar and TCBS agar. RAPD-PCR method was used to identify the genetic variability of the bacteria. Thirty isolates were managed to culture in-vitro. The biochemical tests reveal that, these bacteria can be divided into major group as γ-proteobacteria, ά-proteobacteria, High G+C gram positive bacteria. CFB group and unknown. The bacteria were identified in species level such as *Pantoea dispersa. Pseudomonas sp. Entrobacter agglomerans*, *Cadacea darisae*, *Serratia plymuthica*, *Citrobacter youngae*, *Erwinia herbicola*, *Vibrio sp*, *Klebsielle pneumonia* subspecies *ozanae*, *Aeromonas caviae*, *Alteromonas putrefaciens*, *Serratia sp*, *Alteromonas sp*, *Moraxella sp*, *Photobacterium sp*, *Yersinia bercovieri*, *Vibrio metschnikovii*, *Acinetobacter sp*, *Yersinia entrocolitica*, *Brucella sp*, *Micrococcus sp*, *Micrococcus varians*, *Micrococcus roseus*, *Actinomyces sp* and *Flavobacterium sp*. Many bacteria isolates have demonstrated high levels of genetic variability which the coefficient index shows that bacterial communities in mucus of *A. cervicornis* are in a range of 0 to 1.0. Dendogram reveal the variability of the genetic level. Insufficient data were obtained to support RAPD studies of the bacteria which obtained from mucus.

Pengasingan, Pencirian dan Pengecaman Bakteria yang Berkait Rapat dengan Mucus Terembu Karang(*Acropora cervicornis)* dari Pulau Bidong, Terengganu

ABSTRAK

Pengasingan,pencirian dan pengecaman bakteria daripada mukus karang *Acropora cervicornis* dikutip dari pulau Bidong, Terengganu telah dicapai dengan menggunakan ujian-ujian biokimia dan teknik molekul DNAnya. Bakteria- bakteria ini telah dipencilkan dengan menggunakan kaedah mikrobiologi yang khusus dalam agar 'Sucrose Sea Water (SSW)'. Pencirian morfologi dan fisiologinya telah dilakukan dengan menggunakan pelbagai jenis media seperti MacConkey, Pseudomonas dan TCBS. Kaedah RAPD-PCR digunakan untuk mengetahui kepelbagaian genetik bakteria. Tiga puluh bakteria berjaya dikultur dan diuruskan 'in-vitro'. Ujian-ujian biokimia telah mengkelaskan bakteria ini kepada empat kumpulan utama sebagai ' γ-proteobakteria' , 'ά-proteobakteria', 'High G+C gram positif bakteria', ' kumpulan CFB' dan ada bakteria tidak dapat dikenal pasti kumpulannya. Bakteria yang dikenal pasti sehingga tahap spesis adalah P*antoea dispersa, Pseudomonas sp, Entrobacter agglomerans, Cadacea darisae, Serratia plymuthica, Citrobacter youngae, Erwinia herbicola, Vibrio sp, Klebsielle pneumonia* subspecies *ozanae, Aeromonas caviae, Alteromonas putrefaciens, Serratia sp, Alteromonas sp, Moraxell sp, Photobacteriu sp, Yersinia bercovieri, Vibrio metschnikovii, Acinetobacter sp, Yersinia entrocolitica, Brucella sp, Micrococcus sp, Micrococcus varians, Micrococcus roseus,* and *Actinomyces sp* dan *Flavobacterium sp.* Kebanyakkan bakteria yang dipencil mencirikan tahap kepelbagaian genetik yang tinggi

dimana index koefisian menunjukkan komuniti bakteria dalam mukus *Acropora cervicornis* berada dalam julat 0-1.0. Dendogram menunjukkan kepelbagaian genetik. Walaubagaimanapun, kekurangan data yang diperolehi tidak dapat menyokong kajian RAPD yang diperolehi daripada mukus.