

CONVENTIONAL AND MOLECULAR
CHARACTERIZATION OF *Vibrio parahaemolyticus*, *Vibrio*
alginolyticus AND *Vibrio Vulnificus* FROM OYSTER,
Crassostrea irrorata IN EAST COAST, MALAYSIA

RUHLI HAYATI HAMDAN

MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2008

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Universiti Malaysia Terengganu



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CONVENTIONAL AND MOLECULAR
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alginolyticus AND *Vibrio vulnificus* FROM OYSTER,
Crassostrea iredalei IN EAST COAST, MALAYSIA

RUHIL HAYATI HAMDAN

Thesis Submitted in Full Compliance with the Requirement for the
Degree of Master of Science in Microbiology
Universiti Malaysia Terengganu

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Vibrio alginolyticus AND *Vibrio vulnificus* IN OYSTER,
Crassostrea iredalei FROM EAST COAST, MALAYSIA.**

DEDICATION

*I would like to dedicate this thesis to my supervisor,
abah, mama and my siblings.*

RUHIL HAYATI HAMDAN

Thesis Submitted in Fulfilment of the Requirement for the
Degree of Master of Science in the Faculty of Agrotechnology
and Food Science
Universiti Malaysia Terengganu

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

CONVENTIONAL AND MOLECULAR CHARACTERIZATION OF *Vibrio parahaemolyticus*, *Vibrio alginolyticus* AND *Vibrio vulnificus* FROM OYSTER, *Crassostrea iradelei* IN EAST COAST, MALAYSIA

RUHIL HAYATI RAMDAN

2004

Chairperson : Hajah Masrizah Zakaria, Ph.D.

Member : Ahmad Saad DEDICATION Ph.D.

Faculty : Agricultural and Food Science

***I would like to dedicate this thesis to my supervisor,
abah, mama and my siblings.***

Oyster is a Bivalve mollusc that is widely consumed during important activity. Totally 22, 10 and 16 isolates of *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus* were isolated from oyster, respectively. The morphological, physiological and biochemical studies were done with addition of commercial identification system BBL Crystal™ kit. All isolates were motile Gram-negative, straight and curved rod, non-ligninose, oxidase and catalase positive, sensitive to 1017v (100µg), glucose fermentative, indole fermenting, able to utilize starch, positive for both Caseinase and Lecithinase, and D-lysine. All isolates did not produce H₂S. All isolates also showed negative reactions for Methyl Red, DNPG and urease.

The susceptibility of *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus* against seven antibiotics, namely ampicillin, chloramphenicol, tetracycline, kanamycin, nalidixic acid, tetracycline and streptomycin were determined. The isolates were

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

CONVENTIONAL AND MOLECULAR CHARACTERIZATION OF *Vibrio parahaemolyticus*, *Vibrio alginolyticus* AND *Vibrio vulnificus* FROM OYSTER, *Crassostrea iredalei* IN EAST COAST, MALAYSIA

RUHIL HAYATI HAMDAN

2008

Chairperson : Najiah Musa@Zakaria, Ph.D.
Member : Ahmad Shamsuddin Ahmad, Ph.D.
Faculty : Agrotechnology and Food Science

Oyster is a filter feeder that consumes a large volume of bacteria during ingestion activity. Totally, 28, 10 and 15 isolates of *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus* were isolated from oyster, respectively. The morphological, physiological and biochemical studies were done with addition of commercial identification system BBL Crystal™ Kit. All isolates were motile Gram-negative; straight and curved rod; non-luminous, oxidase and catalase positive; sensitive to O/129 (150µg), glucose fermentative, indole formation, able to utilize starch, positive for both Oxidative and Fermentative, and β-hemolytic. All isolates did not produce H₂S. All isolates also showed negative reactions for Voges Proskauer, ONPG and tetrazolium

The susceptibility of *V. parahaemolyticus*, *V. vulnificus* and *V. alginolyticus* against seven antibiotics; namely ampicilin, chloramphenicol, furazolidone, kanamycin, nalidixic acid, tetracycline and sulphamethoxazole were determined. The isolates were

mostly resistant to ampicillin (90%) followed by Sulphamethoxazole (58%) and furazolidone (44%). However, 100% isolates were demonstrated sensitive to chloramphenicol, tetracycline and nalidixic acid. Furthermore, the isolates were sensitive to kanamycin (90%), furazolidone (56%), sulphamethoxazole (14%) and ampicillin (8%). Intermediate sensitive was observed among the isolates for kanamycin (10%), ampicillin (2%) and sulphamethoxazole (8%). Four different plasmid sizes were detected in *Vibrio* strains ranging from 4 to 54 kb, originating seven different plasmid patterns. However, no particular plasmid profile was extrapolative with particular pattern of antibiotic susceptibility.

In RAPD analysis, the *V. parahaemolyticus*, *V. alginolyticus* and *V. vulnificus* isolates were characterized using a Universal – M13 (5'-TTA TGT AAA ACG ACG GCC AGT-3'), Wild Type Phage – M13 (5'-GAG GGT GGC GGT TCT-3') and T3 (5'-GCA ATT AAC CCT CAC TAA AG-3') - T7 (5'-GTA ATA CGA CGC ACT ATA G-3'). The number of fragments produced per primer ranged from 310 to 360 fragments. The detectable DNA fragments produced by U-M13 wtp-M13 and T3T7 ranged from 250bp to 2500bp, 250bp to 2000bp and 150bp to 2500bp in size, respectively. Both genetic distance and percentage of similarity were inversely correlated. The percentage of similarity among Terengganu isolates ranged from 7 to 100%, while, genetic distance ranged from 0.00 to 0.93. The isolates from Kelantan showed the highest range in percentage of similarity and genetic distance recorded from 0 to 100% and 0.00 to 1.00, respectively. On the other hand, comparison between isolates from Terengganu and Kelantan showed quite high percentage of similarity from 18 to 100%.