



Faculty of Resource Science and Technology

**Prevalence of *Vibrio* spp. Infection in Shrimp and Bio-control Using
Bacteriophages Isolated from Shrimp Farms in Kuching**

Dalene anak Lesen

**Doctor of Philosophy
2024**

Prevalence of *Vibrio* spp. Infection in Shrimp and Bio-control Using
Bacteriophages Isolated from Shrimp Farms in Kuching

Dalene anak Lesen

A thesis submitted

In fulfillment of the requirements for the degree of Doctor of Philosophy

(Molecular Microbiology)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2024

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



.....
Signature

Name: Dalene anak Lesen

Matric No.: 20010016

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date : 5 September 2024

ACKNOWLEDGEMENT

First and foremost, I extend my heartfelt appreciation to my supervisor, Dr. Elexson Nillian, whose keen insight, advice, and unwavering support were indispensable throughout the completion of this study. I am profoundly grateful for his guidance.

My warmest thanks also extend to Dr. Thung Tze Young for his invaluable assistance, constructive feedback, and meticulous review of my work during this study. I am also grateful to have the invaluable guidance from AP Dr. Tan Cheng Siang. Their willingness to help, particularly during moments of uncertainty, was greatly appreciated as it significantly contributed to the quality of this work.

I seize this opportunity to express my heartfelt gratitude to all members of Microbiology 1 Laboratory at the Faculty of Science and Technology. Their unwavering support, shared laughter, experiences, and knowledge have enriched my journey immeasurably, leaving me with cherished memories that I will carry with me always.

Last but certainly not least, I extend a special thank you to my beloved parents, Mr. Lesen anak Jampong and Mdm. Diana @ Tresie anak Charles Brandah, and my family, whose unconditional sacrifices, boundless love, and unwavering encouragement propelled me forward. Additionally, my heartfelt appreciation goes to my friends, whose support has been a constant source of strength throughout my endeavour. Your belief in me and your encouragement during moments of doubt were invaluable. Thank you all from the bottom of my heart.

ABSTRACT

Shrimp farming, a highly profitable sector in global aquaculture, has seen remarkable growth in recent years, with global shrimp consumption projected to reach US\$ 74 billion by 2032. This increasing demand and the expansion of farming operations, including in Sarawak, Malaysia, highlight the sector's potential. However, the industry faces significant challenges, particularly the prevalence of vibriosis, a bacterial infection caused by *Vibrio* species. Contamination of food products has also increased the risk of vibriosis in humans. The widespread use of antibiotics to combat this disease has led to the rapid emergence of antimicrobial resistance (AMR) bacteria. This study presents a comprehensive investigation into the surveillance profiles and bio-control of *Vibrio* spp. isolated from a shrimp farm in Sarawak, Malaysia. A total of 48 (n=48) samples, including water, sediment, shrimp, and effluent, were collected from two ponds throughout the production cycle. The prevalence and quantification of *Vibrio* species were assessed by implementing the MPN-multiplex polymerase chain reaction (PCR) method. The findings revealed high prevalence rates, with *Vibrio parahaemolyticus* being the most prevalent species (97.92%), followed by *Vibrio cholerae* (47.92%) and *Vibrio alginolyticus* (25.0%). The MPN values of *V. parahaemolyticus* were as high as >1,100 MPN/mL or MPN/g in the water, sediment, and shrimp samples of both ponds, which are the highest among the three *Vibrio* species. The MPN values of *V. cholerae* and *V. alginolyticus* remained less than 1,100 MPN/mL or MPN/g in water, sediment, and shrimp samples towards the end sampling period despite starting high initially. Notably, *V. parahaemolyticus* exhibited an increasing trend from stocking to harvesting periods, whereas *V. cholerae* and *V. alginolyticus* showed a decreasing trend. Further analysis involved antibiotic susceptibility testing of 30 (n=30) *Vibrio* spp. isolates by using 18 antibiotics, revealing resistance to at least two antibiotics.

Antibiotics Ceftazidime, Meropenem, Gentamicin, Tetracycline, Nalidixic acid, Norfloxacin, Ciprofloxacin, and Chloramphenicol were 100% effective against all isolates of *V. parahaemolyticus*, *V. cholerae*, and *V. alginolyticus*. Meanwhile, 100% of *V. parahaemolyticus* and *V. alginolyticus* isolates were completely resistant to Penicillin G and Bacitracin, whereas 100% of *V. cholerae* isolates exhibited resistance to Penicillin G. The MAR indices of the isolates ranged from 0.11 to 0.39. In response to the escalating antibiotic resistance, bacteriophages emerged as promising alternatives. Two novel myophages, EniLVP01 and EniLVP02, targeting *V. parahaemolyticus* were isolated, characterised, and found to exhibit narrow host ranges and large burst sizes of 110 and 144 phages per infected cells, respectively. Notably, they effectively prevented and reduced bacterial biofilms. In the biofilm prevention, the absorbances were reduced from 0.592 ± 0.055 to 0.218 ± 0.039 for EniLVP01 and to 0.204 ± 0.016 for EniLVP02. Meanwhile, in the biofilm destruction assay, the mixture treated with the phage lysate of EniLVP01 and EniLVP02 showed an absorbance of 0.139 ± 0.009 and 0.174 ± 0.026 , respectively, compared to the untreated samples with an absorbance of 0.843 ± 0.0029 . Both phages also demonstrated promising efficacy in reducing *V. parahaemolyticus* counts on retail shrimp matrices, with a bacterial reduction of 98% achieved using a cocktail both phages. Phage EniLVP01 and EniLVP02 exhibited stability across a wide range of pH (pH 4.0 – 9.0) and temperature (28 °C - 65 °C) conditions. Genomic sequencing revealed high similarity between EniLVP01 and EniLVP02, suggesting they may belong to the same species in the *Caudovirales* order or are very closely related despite originating from different sources. Importantly, the absence of lysogeny-related, antibiotic, and virulence genes in their genomes supports their safety for therapeutic use. These findings represent a significant advancement in understanding the potential of

phage therapy in the battle against bacterial infections and antibiotic resistance issues within Malaysia generally, and within Sarawak specifically.

Keywords: Vibriosis, antibiotic resistance, phage therapy, bacteriophages, shrimp aquaculture

Kawalan Biologi Terhadap Jangkitan Vibrio Spp. dalam Udang dengan Menggunakan Bakteriofaj yang Diasingkan dari Ladang Udang di Kuching.

ABSTRAK

Akuakultur udang, salah satu sektor yang paling menguntungkan dalam akuakultur global, telah menyaksikan pertumbuhan yang ketara dalam beberapa tahun kebelakangan ini, dengan penggunaan udang global dijangka mencecah AS\$ 74 bilion menjelang tahun 2032. Permintaan yang semakin meningkat dan pengembangan operasi penternakan, termasuk di Sarawak, Malaysia, mengembangkan potensi sektor ini. Namun, industri ini menghadapi cabaran yang besar, khususnya dalam menangani wabak vibriosis, sejenis jangkitan bakteria yang disebabkan oleh spesies Vibrio. Pencemaran pada produk makanan juga telah meningkatkan risiko kejadian vibriosis dalam kalangan manusia. Penggunaan antibiotik yang meluas untuk mengatasi penyakit ini telah menyebabkan kemunculan bakteria rintang antimikrob (AMR) dengan cepat. Kajian ini berkaitan penyelidikan menyeluruh mengenai profil pengawasan dan bio-kawalan Vibrio spp. yang dipencilkan dari ladang udang di Sarawak, Malaysia. Sejumlah 48 (n=48) sampel, termasuk air, endapan, udang, dan sisa kolam, dikumpulkan dari dua kolam sepanjang kitaran pengeluaran udang. Dengan menggunakan kaedah Most Probable Number-reaksi berantai polimerase multiplex (MPN-mPCR), kelaziman dan kuantiti spesies Vibrio dinilai. Penemuan menunjukkan kadar kelaziman yang tinggi, dengan Vibrio parahaemolyticus menjadi spesies yang paling berleluasa (97.92%), diikuti oleh Vibrio cholerae (47.92%) dan Vibrio alginolyticus (25.0%). Nilai MPN V. parahaemolyticus adalah >1,100 MPN/mL atau MPN/g dalam sampel air, endapan, dan udang di kedua-dua kolam, yang merupakan tertinggi di antara ketiga spesies Vibrio. Nilai MPN V. cholerae dan V. alginolyticus kekal kurang daripada 1,100 MPN/mL atau MPN/g dalam sampel air, endapan, dan udang menjelang akhir tempoh

pensampelan walaupun bermula tinggi pada awalnya. Selain itu, V. parahaemolyticus menunjukkan tren peningkatan dari tempoh penyimpanan stok hingga tempoh menuai, manakala V. cholerae dan V. alginolyticus menunjukkan tren penurunan. Analisis lanjut melibatkan ujian kepekaan antibiotik terhadap 30 (n=30) isolat Vibrio spp. mendedahkan ketahanan terhadap sekurang-kurangnya dua antibiotik. Antibiotik Cefazidime, Meropenem, Gentamicin, Tetracycline, Nalidixic acid, Norfloxacin, Ciprofloxacin, dan Chloramphenicol adalah 100% berkesan terhadap semua isolat V. parahaemolyticus, V. cholerae, dan V. alginolyticus. Sementara itu, 100% isolat V. parahaemolyticus dan V. alginolyticus adalah tahan sepenuhnya kepada Penicillin G dan Bacitracin, manakala 100% isolat V. cholerae menunjukkan ketahanan kepada Penicillin G. Indeks MAR isolat berjulat dari 0.11 hingga 0.39. Sebagai penyelesaian terhadap peningkatan ketahanan antibiotik di kalangan bacteria, bakteriofaj menunjukkan pontesinya sebagai alternatif untuk antibiotik. Dua myofaj yang baru, EniLVP01 dan EniLVP02, yang berupaya memusnahkan V. parahaemolyticus diasingkan, dicirikan, dan didapati menunjukkan julat hos yang kecil dan saiz pemecahan yang besar masing-masingnya 110 dan 144 faj per sel terinfeksi. Faj-faj ini mengurangkan dan mencegah biofilem bacteria secara berkesan. Dalam pencegahan biofilm, penyerapan berkurang dari 0.592 ± 0.055 kepada 0.218 ± 0.039 untuk EniLVP01 ($P < 0.05$) dan kepada 0.204 ± 0.016 untuk EniLVP02. Sementara itu, dalam ujian pemusnahan biofilem, sampel yang dirawat dengan lisat faj EniLVP01 dan EniLVP02 menunjukkan penyerapan 0.139 ± 0.009 ($P < 0.05$) dan 0.174 ± 0.026 ($P < 0.05$) berbanding dengan sampel yang tidak dirawat yang mempunyai penyerapan 0.843 ± 0.0029 . Selain itu, kedua-dua faj juga menunjukkan keberkesanan yang memberangsangkan dalam mengurangkan bilangan V. parahaemolyticus pada matriks udang, dengan pengurangan bacteria sehingga 98% menggunakan koktel faj. Faj EniLVP01 dan EniLVP02,

menunjukkan kestabilan melangkaui julat pH (pH 4.0 - 9.0) dan suhu (28 °C - 65 °C) yang tinggi. Analisa genomik mendedahkan kesamaan tinggi antara EniLVP01 dan EniLVP02, mencadangkan kedua-dua faj ini mungkin spesies yang sama dalam order Caudovirales atau sangat berkaitan meskipun berasal dari sumber yang berbeza. Ketiadaan gen berkaitan lizogeni, antibiotik, dan virulensi pada genom faj EniLVP01 dan EniLVP02 menyokong keselamatan dalam penggunaan terapeutik. Penemuan ini menunjukkan kemajuan penting dalam memahami potensi terapi faj bagi melawan jangkitan bakteria dan isu ketahanan antibiotik di Malaysia umumnya dan di Sarawak khususnya.

Kata kunci: *Vibriosis, ketahanan antibiotik, terapi faj, bakteriofaj, akuakultur udang*

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistant
ARG	Antibiotic resistance gene
MGE	Mobile genetic element
MDR	Multi drug resistant
APW	Alkaline peptone water
TCBS	Thiosulfate-citrate-bile salts-sucrose
MPN	Most Probable Number
mPCR	Multiplex Polymerase Chain Reaction
PFU	Plaque forming unit
CFU	Colony forming unit
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
ORF	Open reading frame
CDS	Coding sequence
NGS	Next generation sequencing
WGS	Whole genome sequencing