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# MOLECULAR DETECTION OF *Lux*R HOMOLOGUE GENE IN *VIBRIO* SP.

By

**NITHIANANTHA A/L JEYAKANTHAN**

A RESEARCH PAPER SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE

FACULTY OF VETERINARY MEDICINE

UNIVERSITI MALAYSIA KELANTAN

2025

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**Molecular detection of *Lux*R homologue gene from *Vibrio* sp**. This research paper, presented to the Faculty of Veterinary Medicine at Universiti Malaysia Kelantan, represents a crucial step in advancing our understanding of pathogenic microorganisms within aquatic environments. It fulfills a partial requirement for the course DVT 55204 - Research Project, which emphasizes the significance of research in veterinary medicine.



## ABSTRACT

The *Lux*R homologue gene plays a central role in quorum-sensing regulation among *Vibrio* species, influencing virulence, biofilm formation, and host–pathogen interactions. This study aimed to detect the presence of *Lux*R homologue genes in three *Vibrio* isolates obtained from aquatic environments using molecular techniques. Genomic DNA was extracted from each isolate, and PCR amplification was conducted using *Lux*R-specific primers targeting conserved regulatory regions. None of the three *Vibrio* isolates produced the expected amplicon corresponding to the *Lux*R homologue gene. The absence of *Lux*R-related sequences suggests that these isolates may rely on alternative quorum-sensing pathways, possess divergent *Lux*R-like regulators not amplified by the primers used, or belong to *Vibrio* species lacking conventional *Lux*R regulatory elements. These findings highlight the diversity of quorum-sensing mechanisms within *Vibrio* spp. and underscore the need for broader genomic screening or primer optimization in future studies.

Keywords: *Vibrio* sp, *Lux*R homologue gene, Polymerase Chain Reaction,

## ABSTRAK

Gen homologi *Lux*R memainkan peranan penting dalam pengawalan quorum sensing dalam kalangan spesies *Vibrio*, mempengaruhi virulens, pembentukan biofilm, dan interaksi antara hos dan patogen. Kajian ini bertujuan mengesan kewujudan gen homologi *Lux*R dalam tiga isolat *Vibrio* yang diperoleh daripada persekitaran akuatik menggunakan kaedah molekul. DNA genomik telah diekstrak daripada setiap isolat, dan penguatan PCR dijalankan menggunakan primer khusus *Lux*R yang menyasarkan kawasan regulatori terpelihara. Tiada satu pun daripada tiga isolat *Vibrio* menghasilkan amplicon yang dijangka sepadan dengan gen homologi *Lux*R. Ketiadaan jujukan berkaitan *Lux*R mencadangkan bahawa isolat tersebut mungkin bergantung pada laluan quorum sensing alternatif, mempunyai pengawal selia mirip *Lux*R yang berbeza dan tidak dapat diamplifikasi oleh primer yang digunakan, atau tergolong dalam spesies *Vibrio* yang tidak mempunyai elemen regulatori *Lux*R konvensional. Penemuan ini menonjolkan kepelbagaian mekanisme quorum sensing dalam *Vibrio* spp. dan menggariskan keperluan untuk saringan genom yang lebih luas atau pengoptimuman primer dalam kajian masa hadapan.

## ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest gratitude to God for His blessings, strength, and guidance throughout the completion of this research project. I would like to extend my heartfelt appreciation to Dr. Ruhil Hayati binti Hamdan, my supervisor, for her continuous support from the proposal preparation stage until the completion of this thesis. Thank you, Dr., for teaching me essential technical skills such as preparing agar and culturing bacteria. These skills greatly eased my research work and will be invaluable for my future scientific endeavors. My sincere thanks also go to Dr. Tan Li Peng, my co-supervisor, for her guidance, advice, and encouragement throughout this project. I would like to express my appreciation to Encik Safuan for his assistance with bacterial culture work in the aquatic laboratory. My gratitude also goes to Cik Salma for her support and guidance during the molecular detection process. I have learned many important laboratory skills from her, which greatly contributed to the progress of this study. Finally, I would like to thank my family for their continuous support, patience, and encouragement throughout my journey in the DVM course. Their unwavering belief in me has been my greatest source of strength.

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**List of abbreviations**

QS- Quorum sensing

PCR-Polymerase Chain Reaction

TSA- Tryptine Soy Agar

TSB- Tryptine Soy Broth

AHLs- Acyle-hemoserine lactones RNA- Ribonucleic acid

DNA- Deoxyribonucleic acid sRNAs- Small RNA

MG- Magnesium Cl- Chlorine

DNTP- Deoxyribonucleoside triphosphate BP- Base Pair

# CHAPTER 1

## INTRODUCTION

*Vibrio spp a*re Gram-negative, rod-shaped bacteria, that are found naturally in freshwater, estuaries, and marine habitats. These bacteria are native to aquatic and marine habitats, like warm, brackish water, and their abundance in the wild often reflects the temperature of the surrounding environment (Baker-Austin et al., 2018). Vibriosis is the collective term for diseases that can cause serious human outbreaks when contaminated water or seafood containing *Vibrio* spp. is consumed (Thomas, et all. 2023). A wide variety of aquatic animals, such as fish, crabs, and mollusks, have been affected by *Vibrio* spp*.* (Souza Valente et al., 2021). Numerous *vibrio* spp. have been identified as the causative agents of vibriosis in the aquaculture sector, which has resulted in significant financial losses (Kyle et al., 2021). Over 20 more than 80 species of *Vibrio* microbes are capable of causing vibriosis and the common species responsible for vibriosis are *Vibrio parahaemolyticus* and *Vibrio Vulnificus* (Andrew et al., 2021). An established pathway of human exposure is the buildup of vibrios in shellfish, especially bivalves, and subsequent ingestion of those products either raw or incompletely cooked (Konstantinos Koutsoumanis et al., 2024). Thus, most of the human exposure to vibriosis is occurring by consumption of infected seafood with pathogenic *Vibrio* spp. PCR is a molecular technique that has been used to identify foodborne bacteria and screen for particular genes linked to pathogenicity by focusing on particular genes of various Vibrio species that cause vibriosis. *Vibrio* species such as *V. cholerae, V. parahaemolyticus*, *V. harveyi*, V.alginolyticus, V.anguillarum can be identified using these these PCR assays (Keyan-Loo, et al., 2022). This

technique helps process more samples and identify the causative agent quickly and at a low cost. A key regulatory mechanism in *Vibrio* pathogenesis and environmental adaptation is quorum sensing (QS) - a population density-dependent system controlling gene expression through signal molecules. The *LuxR* family of transcriptional regulators is central to QS systems. *Lux*R homologues, in combination with their corresponding autoinducers (like acyl-homoserine lactones), coordinate the expression of genes involved in virulence, biofilm formation, and motility (Waters & Bassler, 2005). Despite the significance of *Lux*R-type regulators, studies on their molecular detection and distribution among *Vibrio* spp. in Malaysian aquatic environments remain limited.

## 1.1 RESEARCH PROBLEM STATEMENT

While quorum sensing has been well-documented in model *Vibrio* species such as *V. harveyi* and *V. fischeri*, the presence and diversity of *Lux*R homologue genes in locally isolated *Vibrio* spp. are underexplored. This gap limits our understanding of the molecular mechanisms underlying virulence regulation and environmental adaptability in these bacteria. There is a need for molecular-based detection to characterize *Lux*R homologues and to assess their prevalence in environmental or clinical *Vibrio* isolates.

## 1.2 RESEARCH QUESTION

Are *Lux*R homologue genes present in *Vibrio* spp. isolated from aquatic environments?

## 1.3 RESEARCH HYPOTHESIS

*Lux*R homologue genes present in *Vibrio* spp. isolated from aquatic environments.

## 1.4 RESEARCH OBJECTIVES

To detect the presence of *Lux*R homologue genes in *Vibrio* spp. isolates using PCR-based molecular techniques.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Quorum signaling.

Quorum sensing is the method by which bacterial gene expression is controlled by adaptable chemical signaling molecules called auto inducers, which are essential for bacterial communication (Windsor, 2020). In addition, quorum sensing controls a wide range of bacterial processes, such as biofilm formation and pathogenicity (Lantian et al., 2020). As a result, the quantum sensing system is essential for coordinating the expression of genes linked to different Vibrio phenotypes and virulence traits (V.N Lydick et al., 2025).

## 2.2 Quorum signaling system of gram-negative bacteria.

For Gram-negative bacteria the quorum sensing system consists of *Lux*I or *Lux*R (YingPing et al., 2022**)**. Gram-negative bacteria's *Lux*R-type receptors can be classified as either *Lux*R solo or Orphan and typical *Lux*R-type receptors. *Lux*I synthase produces acyl-homoserine lactones (AHLs), which are detected by a class of proteins known as typical *Lux*R-type receptors. For example, typical *Lux*R-type receptors are derived from the *Lux*R protein found in Vibrio fischeri After detecting and binding AHLs (N-3-(oxo-hexanoyl)-homoserine lactone) ligands made by

*Lux*I proteins, it triggers the luciferase operon's transcription (Li Yi et al., 2020). *Vibrio. harveyi* is an excellent model organism for studying quorum sensing pathways in other vibrios. Similar structures have been identified, including membrane-bound autoinducer receptors, short regulatory RNAs (sRNAs), and *Lux*R homologs serving as main regulation.

2.3 *LuxR* homologue gene.

*Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus,* and *Vibrio alginolyticus* are among them (Alyssa et al., 2017). Through *Lux*R sequence analysis in PCR, which involves preparing and amplifying the desired genes in PCR and then visualizing them in agarose gel electrophoresis, the *Lux*R homologous gene can be detected (Tang et al., 2019). Quorum sensing is a critical regulatory system in many bacterial species, including *Vibrio*, where it modulates genes essential for biofilm formation, bioluminescence, and virulence (Miller & Bassler, 2001). The *Lux*R family comprises transcriptional regulators that, when bound to autoinducers, activate or repress target genes (Defoirdt et al., 2005).Studies have shown that *Lux*R homologues are structurally conserved but functionally diverse across *Vibrio* spp. (Zhu & Winans, 2001).

2.3 Molecular detection

Molecular detection of *Lux*R-like genes using PCR primers targeting conserved domains has been successfully employed in environmental surveillance (Cao & Meighen, 1989

# CHAPTER 3

# RESEARCH METHODOLOGY

## 3.1 Bacterial Isolation

Total 6 of *Vibrio* isolates were obtained from archive samples stored in the Trypticase Soy Broth, TSB with 50% glycerol stocks in -80˚C freezer in Zoonotic Laboratory, Faculty of Veterinary Medicine, Universiti Malaysia Kelantan. The isolated samples were B7 5/5, IP3 1/2, 1P7, 1P8, K2 17, and K2 16. These isolates were revived on TSA with 2% NaCland incubated at 30°C for 24h..

## 3.2 DNA extraction

Only three samples IP3, K2 17, and K2 16 showed pure, isolated single colonies, whereas the remaining samples had overgrown colonies, likely due to contamination. Therefore, only IP3, K2 17, and K2 16 were selected for DNA extraction and K2 17 and K2 16 were repeated twicely for DNA extraction. Genomic DNA from these samples was extracted using the boiling method described by García-Aljaro et al. (2012). Single colonies were inoculated into 20 µl of double-distilled water and boiled for 10 minutes. The samples were then chilled and centrifuged for 5 minutes at 16,000 g. Finally, 5 µl of the resulting supernatant from each sample was used as the template for PCR.

Table 1: *Vibrio* spp used for DNA extraction.

| **Sample ID** | ***Vibrio* spp** |
|---|---|
| IP3 | *Vibrio Xuii* |
| K2 17 | *Vibrio rotiferianus* |
| K2 16 | *Vibrio hepatitis* |

3.3 PCR amplification of *Lux*R Homologue Gene

In this research, *LuxRI-F2* and *LuxRI-R2* were used to amplify *Lux*R gene from the obtained samples.These primers were used in previous studies to amplify *Lux*R gene from *Vibrio harveyi* and other *Vibrio* species (Pujalte et al., 2012). The reagents and volume used for a single PCR reaction which included master mix (PCR buffer, Mg+, Cl-, DNTP, Taq polymerase) with the volume of 12.5 µ$l$, forward primer 1 µ$l$, reverse primer 1 µ$l$, nuclease free water 8.5 µ$l$, and DNA template 5 µ$l$. The reaction was done in T100 Thermal Cycler (Bio-Rad, US).

Table 2: Primers used for amplification of the *Lux*R homologue gene

| Gene | Primers | DNA sequence | Protocol | Product size |
|---|---|---|---|---|
| *Lux*R | *LuxRI-F2* | 5′-ATGAAAGTTG ATGAAAGTGG-3′ | Initial denaturation at 95°C for 5 min; 35 cycles of denaturation (95°C, 30s), annealing (55–60°C, 30s), and extension (72°C, 1 min); final extension at 72°C for 10 min. | -635 bp |
| | *LuxRI-R2* | 5′-TTAGTTGTTG ATCGCGTTGG-3′ | | |

3.4 Gel Electrophoresis and Visualization

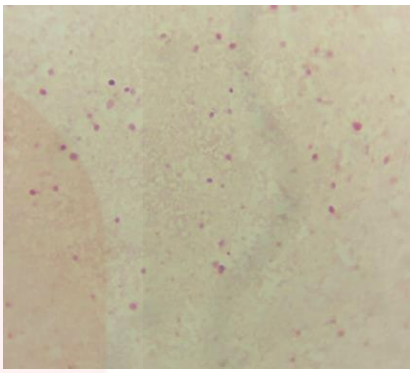
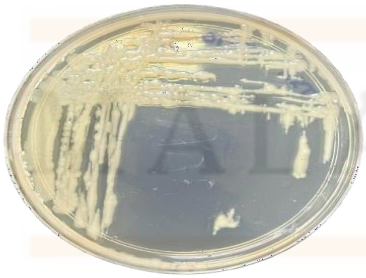
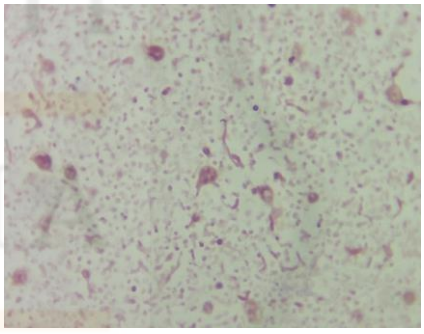
PCR products were run on 1.5% agarose gel stained with GelRed. The gel was visualized under UV transillumination.

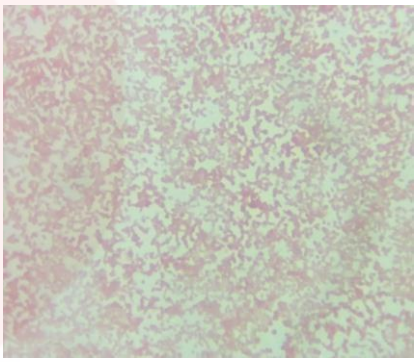
# CHAPTER 4

# RESULTS

## 4.1 Bacterial isolation and Identification

Table 3: Bacterial culture and Gram-staining

| Sample | Colony morphology | Gram-staining |
|---|---|---|
| IP3 |  <br>• White, round, opaque and smooth colonies |  <br>• Gram- negative, curved rod-shaped bacilli |
| K2 17 |  <br>• Yellow, round, opaque, and smooth colonies |  <br>• Gram-negative, rod-shaped bacilli |

| | | |
|---|---|---|
| K2 16 |  <br>• Yellowish confluent, round, opaque and smooth colonies |  <br>• Gram- negative, rod-shape bacilli |

4.2 Biochemical test

Table 4: Oxidative l test

| Sample | Result |
|---|---|
| IP3 | Positive |
| K2 17 | Positive |
| K2 16 | Positive |

4.3 Gel electrophoresis

Gel electrophoresis visualization of the PCR products from samples IP3, K2 17, and K2 16 showed an absence of a visible band at the predicted size for the amplified *Lux*R gene. This finding confirms the absence of the *Lux*R gene in the isolated *Vibrio* sp. sample obtained from Zoonotic lab.

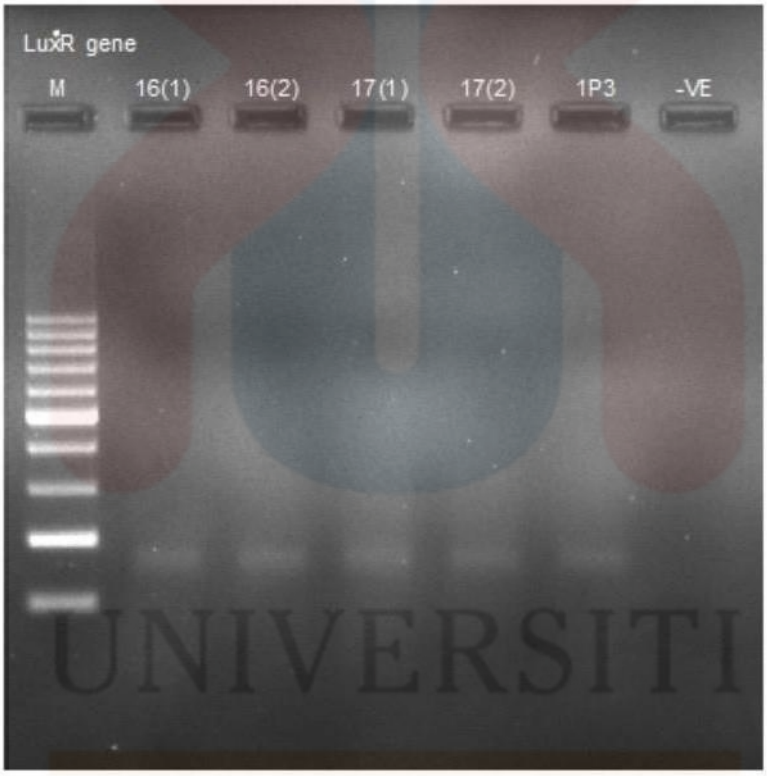



Figure 1: Result of Gel electrophoresis

# CHAPTER 5

## DISCUSSION

The *Lux*R gene was not successfully amplified from the three Vibrio isolates (IP3, K2 17, and K2 16) that generated pure colonies in the current research. The lack of amplification indicates that these isolates might not have the particular *Lux*R homologue targeted by the primer set employed, even though the discovery of *Lux*R was expected based on prior discoveries in several Vibrio species. This result reflects the possible genetic heterogeneity among strains isolated from various environments and emphasises the complexity and diversity of quorum-sensing systems within the genus *Vibrio* (Adam et al., 2005).

*Vibrio* spp. are known to utilise various quorum-sensing pathways including many regulatory components. While the conventional *LuxR*-based system has been thoroughly researched in model organisms such as *Vibrio harveyi* and *Vibrio fischeri,* not all Vibrio species rely on this same regulatory structure (Christopher & Bonnie, 2006). The bacteria are able to coordinate population- dependent behaviors including pathogenicity, biofilm formation, and bioluminescence because the *Lux*R gene encodes a transcriptional regulator that reacts to auto inducer signals. The presence, sequence, and function of *Lux*R homologues, however, differ significantly among Vibrio species, according to genomic research. Some strains possess considerably different variants of the gene, while others lack the original form altogether.

Some *Vibrio* species rely more on alternative quorum-sensing regulators, such as *Lux*O*, Lux*N, cqsS, or other *Lux*R family members having little sequence similarity to the primers used in this investigation, rather than the conventional *Lux*R (Lisa et al., 2016). Even if a functional have a

higher chance of retaining viability and intact genetic material, which raises the possibility of successful gene identification and lowers the possibility of false negatives brought on by deteriorated DNA in samples that have been preserved.

Technical techniques should also be extensively refined. Contamination concerns can be decreased by enforcing strict sample handling procedures, increasing pipetting accuracy, and optimising bacterial culturing techniques to provide ideal growth circumstances. These changes not only enhance the reliability and reproducibility of outcomes but also increase overall experimental efficiency. By addressing both biological and technical limitations observed in the current study, future research can generate more accurate insights into *Lux*R-mediated quorum sensing, virulence regulation, and gene networking in Vibrio species, ultimately contributing to better disease management strategies in aquaculture and public health.

This study investigated the presence of the LuxR homologue gene, a key quorum-sensing regulator, in three Vibrio isolates using PCR-based molecular detection. Despite successful genomic DNA extraction and appropriate positive controls, none of the isolates produced the expected LuxR amplicon. These results suggest that the isolates may lack the canonical LuxR homologue gene, possess LuxR-like regulators with significant sequence divergence, or rely on alternative quorum-sensing pathways independent of LuxR. The absence of LuxR detection highlights the genetic diversity within Vibrio spp. and indicates that LuxR-based screening alone may not fully represent quorum-sensing capabilities or virulence potential among environmental Vibrio isolate

# CHAPTER 6

## CONCLUSION AND RECOMMENDATION

In the *Vibrio* species, the *Lux*R protein is a transcriptional regulator that reacts to signals from quorum sensing. Quorum sensing allows bacteria to monitor their population density through chemical signalling molecules called autoinducers. When the population hits a certain threshold, *Lux*R activates or represses specific virulence genes, including those responsible for toxin synthesis, secretion systems, and adhesion factors. By controlling these virulence proteins, *Lux*R enables the bacteria to coordinate the timing of infection, maximising their survival and pathogenicity. Comprehending this mechanism offers a framework for managing bacterial virulence without necessarily eliminating the bacteria, which is especially helpful for creating anti-virulence treatments and lowering the likelihood of antibiotic resistance.

Bacteria must continually adjust to dynamic surroundings, including shifts in food availability, temperature, salinity, and host immunological defences. LuxR-mediated quorum sensing allows Vibrio spp. to "sense" their surroundings and alter gene expression accordingly. For example, under stress or low nutritional conditions, *Lux*R can trigger genes associated in motility, biofilm formation, and metabolic changes. This adaptive behaviour guarantees that the bacterial population can endure adverse conditions, colonise new niches, and outcompete other microbes. Complete understanding of these adaptive methods also provides insight into bacterial resilience and persistence in both natural and artificial habitats.

Altering with *Lux*R signalling can stop bacteria from coordinating infection because *Lux*R regulates the production of virulence proteins in a population-density-dependent way. This strategy is termed quorum quenching. It is feasible to lessen pathogenicity without applying the selective pressure that causes antibiotic resistance by focussing on *Lux*R or its signalling pathways. In aquaculture, for example, interrupting LuxR-regulated communication can lessen outbreaks of Vibrio-associated illnesses in fish and shellfish. Similarly, in human health, blocking *Lux*R pathways could minimise infections caused by harmful Vibrio species, offering a potential preventive method that complements established antibiotic medicines.

Structured bacterial populations that are affixed to surfaces and enmeshed in an extracellular matrix that they manufacture themselves are known as biofilms. By controlling genes related to adhesion, matrix synthesis, and cell aggregation, LuxR plays a critical role in the formation of biofilms. Bacteria are shielded by biofilms from antimicrobial agents, host immunological reactions, and environmental stress. Researchers can find possible solutions to stop persistent infections, manage bacterial survival in aquatic systems, and minimize industrial bio fouling by examining how *Lux*R controls biofilm development. In order to understand chronic infections, where biofilms serve as reservoirs for disease recurrence, it is also essential to appreciate LuxR-mediated biofilm dynamics.

It is recommended that more bacterial isolates should be investigated in subsequent research on the L*ux*R gene in Vibrio species. A higher sample size would provide a more comprehensive understanding of the prevalence and distribution of *Lux*R among different Vibrio strains, boosting the statistical robustness of the findings. Additionally, using newly separated samples directly from fish hosts rather than long-stored or frozen samples is encouraged. Fresh isolates

have a higher chance of retaining viability and intact genetic material, which raises the possibility of successful gene identification and lowers the possibility of false negatives brought on by deteriorated DNA in samples that have been preserved.

Technical techniques should also be extensively refined. Contamination concerns can be decreased by enforcing strict sample handling procedures, increasing pipetting accuracy, and optimising bacterial culturing techniques to provide ideal growth circumstances. These changes not only enhance the reliability and reproducibility of outcomes but also increase overall experimental efficiency. By addressing both biological and technical limitations observed in the current study, future research can generate more accurate insights into *Lux*R-mediated quorum sensing, virulence regulation, and gene networking in Vibrio species, ultimately contributing to better disease management strategies in aquaculture and public health.

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**Recommendations**

1. **Use of Degenerate or Redesigned Primers**

   Primer mismatch may have contributed to non-amplification. Future studies should test degenerate primers or target more conserved *Lux*R domains to improve detection sensitivity.

2. **Expand the Number of Isolates**

   Screening a larger panel of *Vibrio* isolates from different environments (marine, estuarine, aquaculture water) will provide a more comprehensive understanding of *Lux*R distribution.

3. **Include Additional Quorum-Sensing Genes**

   Since *Vibrio* species may rely on multiple QS regulators, future work should examine genes such as *hapR*, *opaR*, *smcR*, *aphA*, *luxO*, or *qrr* small RNAs to map alternative QS pathways.

4. **Perform Whole-Genome Sequencing**

   Genomic analysis will help identify non-canonical *Lux*R-like regulators or QS components not detectable through targeted PCR.

5. **Assess Phenotypic Traits Related to QS**

   Testing biofilm formation, motility, hemolytic activity, or virulence factors may clarify whether *Lux*R-independent QS pathways are functional in these isolates.

6. **Optimise PCR Conditions**

   Adjusting annealing temperatures, $Mg^{2+}$ concentration, and cycling conditions can help overcome weak or divergent target sequences.

**Appendices**

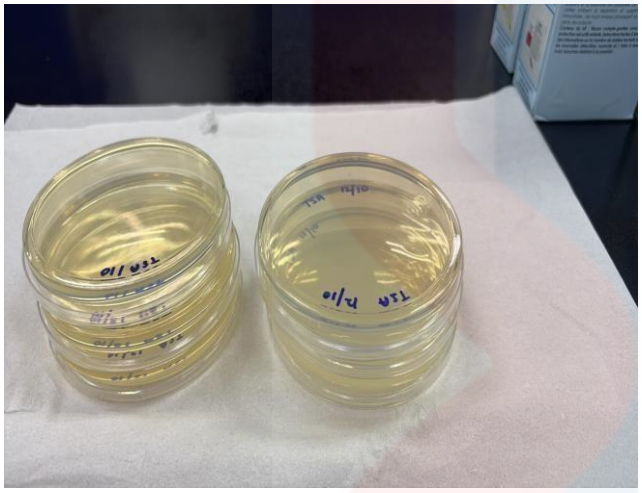

Figure 1: Preparation of TSA agar

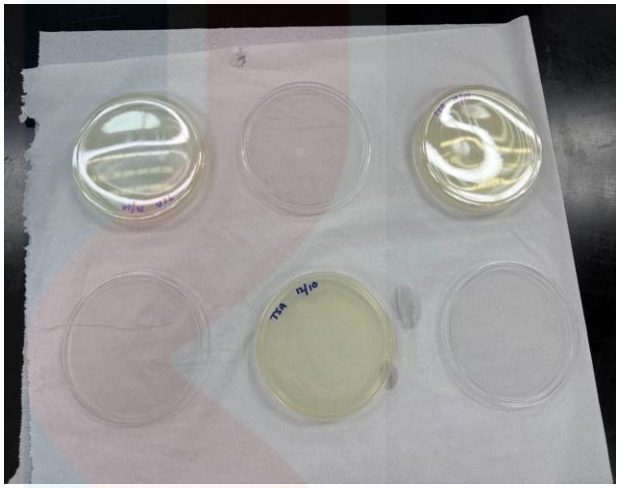

Figure 2: Drying TSA agar

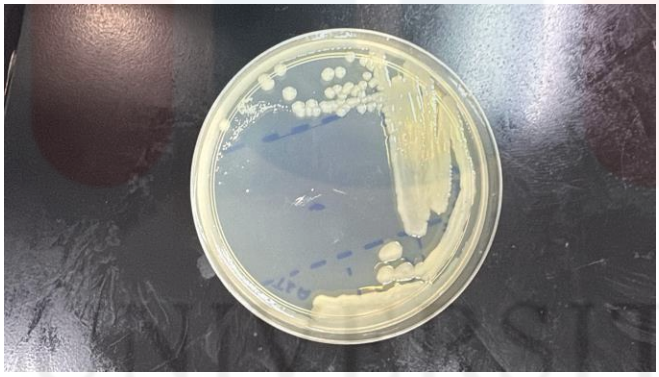

Figure 3: Growth of *Vibrio* sp colonies on TSA

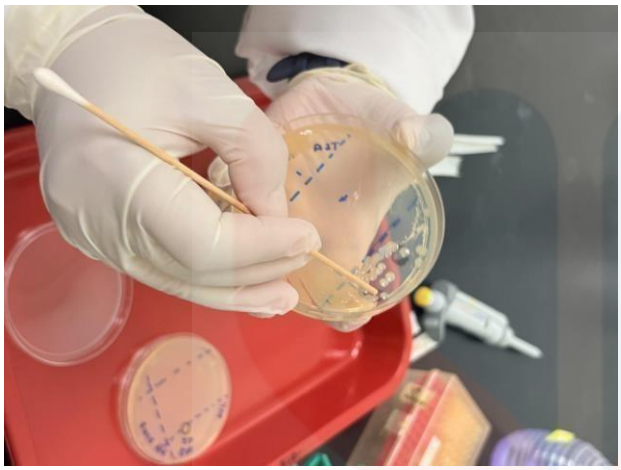

Figure 4: Taking single colony

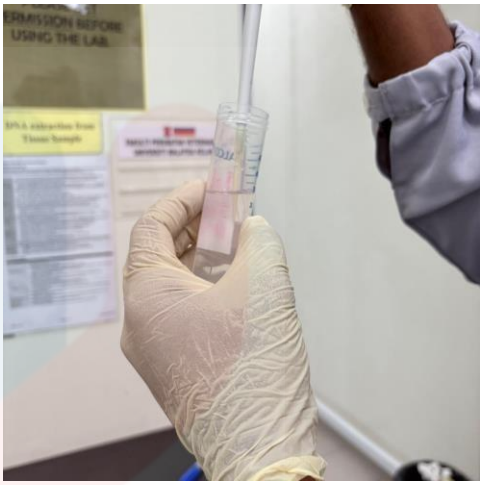

Figure 5: Preparing reaction mixture

Figure 6: Adding DNA sample


Figure 7: Spinning the PCR tube

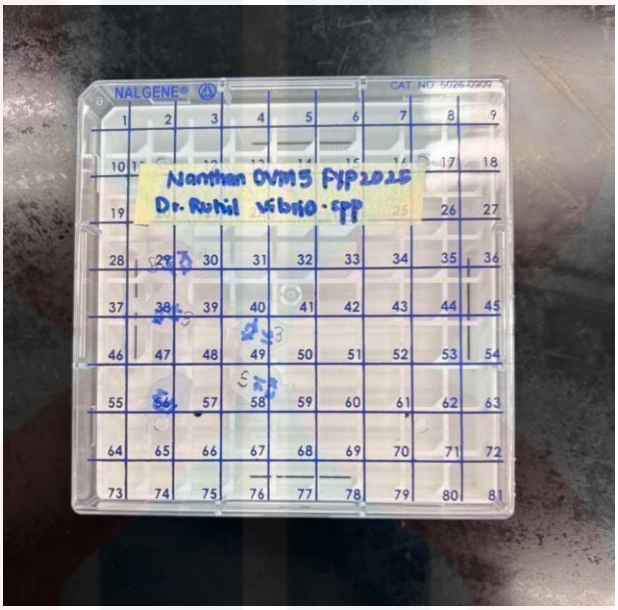


Figure 8: Extracted DNA

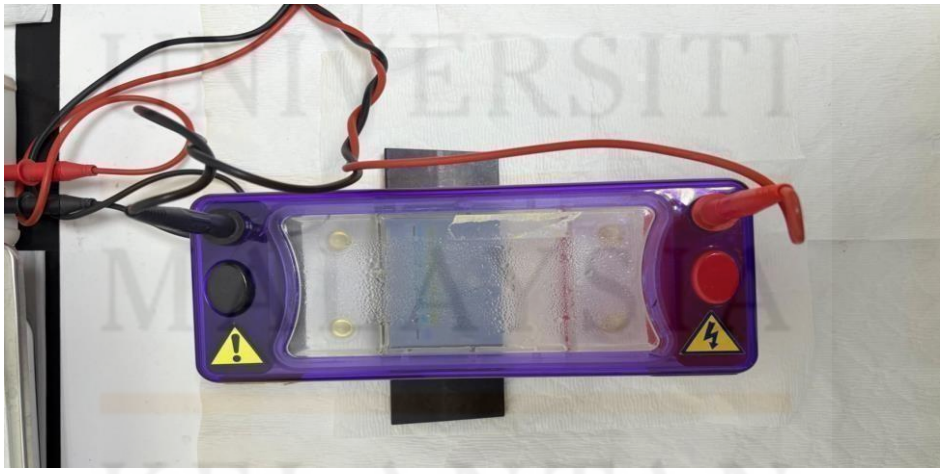

Figure 9: Running PCR product in gel electrophoresis

**References**


1. Andrew Bell, Michael Bott, (2021). Vibriosis. https://pmc.ncbi.nlm.nih.gov/articles/PMC8352540/pdf/djph-71-005.pdf

2. Alif Haikal Mazlan, muhammad Hafizal Aqhmal Muhamad Najib, Mizaton Hazizul Hassan, Fazleen Haslinda Mohd Hatta, Rosmadi Mohd Yusoff.(2024).Effect of DNA Template Concentration on Standard Polymerase Chain Reaction. 10.24191/IJPNaCS.v7i1.01

3. Alyssa S.Bali, R.Chaparian, C.van Kessel, (2017). Quorum Sensing Gene Regulation by *Lux*R/*Hap*R Master Regulators in Vibrios, https://journals.asm.org/doi/epub/10.1128/jb.00105-17

4. Adam Joelsson, Zhiu Liu,Jun Zhu.(2005).Genetic and Phenotypic Diversity of Quorum-Sensing Systems in Clinical and Environmental Isolates of *Vibrio cholerae*. https://pmc.ncbi.nlm.nih.gov/articles/PMC1360356/pdf/1523-05.pd

5. Baker-Austin,D.Oliver, Munirul Alam, Afsar Ali, K.Waldor, Firdausi Qadri, Martinez- Urtaza (2018). Vibrio spp infections,1-19. https://www.nature.com/articles/s41572-018-0005-8

6. Cao, J. G., & Meighen, E. A. (1989). Purification and structural identification of an autoinducer for the luminescence system of Vibrio harveyi. Journal of Biological Chemistry, 264(36), 21670–21676.

7. Defoirdt, T., Boon, N., Sorgeloos, P., Verstraete, W., & Bossier, P. (2005). Quorum sensing and quorum quenching in *Vibrio harveyi:* lessons learned from in vivo work. The ISME Journal, 1(1), 19–26.

8. García-Aljaro, C., Melado-Rovira, S., Milton, D.L. et al. Quorum-sensing regulates biofilm formation in Vibrio scophthalmi. BMC Microbiol 12, 287 (2012). https://doi.org/10.1186/1471-2180-12-28

9. Garcia-Aljaro C, Muniesa M, Jofre J, Blanch AR: Prevalence of the stx2 gene in coliform populations from aquatic environments. Appl Environ Microbiol. 2004, 70 (6): 3535-3540. https://doi.org/10.1128/AEM.70.6.3535-3540.2004

10. Jung-Lim Lee,Robert E.Levin.(2009). Discrimination of viable and dead Vibrio vulnificus after refrigerated and frozen storage using EMA, sodium deoxycholate and real-time PCR. https://doi.org/10.1016/j.mimet.2009.08.014

11. Ke-Yan Loo, Fei Law, Hern Tan, P. Pusparajah, V.Letchumanan, Han Lee, (2022).Diagnostic techniques for rapid detection of *Vibrio* spp. https://doi.org/10.1016/j.aquaculture.2022.738628

12. Koutsoumanis, Allende, Ordonez, Bolton, Cid, Chemaly,De Cesare, Herman, Hilbert, Lindqvisit,Nauta, Nonno, Peixe, Giuseppe Ru, Simmons, Skandamis, Austin, Hervio, Urtaza, Caro, Strauch, Thebault, Guerra, Messens, Simon, Barcia, Suffredini, (2024). Public health aspect of Vibrio spp. related to the consumption of seafood in theEU. https://doi.org/10.2903/j.efsa.2024.8896

13. Kyle D., Moiz Usmani, M. Chen, Mayank Gangwar, S.Jutla, Anwar Huq, R. Colwell ( 2021).Environmental parameters associated with incidence and transmission of pathogenic *Vibrio* spp, 7313-7340.https://doi.org/10.1111/1462-2920.15716

14. Lantian Zhou, Yue Zhang, Yongze Ge, Xuan Zhu, Jianyi Pian, ( 28 January 2021).Regulatory Mechanisms and Promising Applications of Quorum Sensing-Inhibiting Agents in Control of Bacterial Biofilm Formation,

15. Lisa A.Hawver, Sarah A.Jung,Wai-Leung Ng.(2016).Specificity and complexity in bacterial quorum-sensing systems.https://doi.org/10.1093/femsre/fuw014

16. Li Yi, X. Dong, D. Grenier, Wang, Y. Wang, (2020). Research progress of bacterial quorum sensing receptors: Classification, structure, function and characteristics,12 https://sci-hub.se/https://www.sciencedirect.com/science/article/abs/pii/S004896972036561X

17. Leo Cordsmeier, Marc Benjamin Hahn.(2022).DNA Stability in Biodosimetry, Pharmacy and DNA Based Data-Storage: Optimal Storage and Handling Conditions. https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cbic.202200391

18. Miller, M. B., & Bassler, B. L., (2001). Quorum sensing in bacteria. Annual Review of Microbiology, 55(1), 165–199.

19. Nicola Lorenz, Jae Yen Jin, Kristen Jeng, (April, 2017). Activity, Abundance, and Localization of Quorum Sensing Receptor in Vibrio harveyi. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2017.00634/full

20. R.Tang, J. Zhu, Feng, J. Li, X. Liu, (2019). Characterization of *Lux*L/*Lux*R and their regulation involved in biofilm formation and stress resistance in fish spoilers Pseudomonas fluorescens, 60-71 S016816051830

21. R Sepp.I Szabo,H Uda, H Sakamoto. (1994). Rapid techniques for DNA extraction from routinely processed archival tissue for use in PCR. https://pmc.ncbi.nlm.nih.gov/articles/PMC501934/pdf/jclinpath00217-0036.pdf6901

22. Souza Valente, Alex H.L Wan, (2021). *Vibrio* and major commercially important vibriosis diseases in decapod crustaceans, 1 S0022201120302330\

23. Shuyang Hue, Yuwei Li, Boran Wang, Lijun Yin, Xin Jia, (March, 2022). Effects of NaCl Concentration on the Behavior of Vibrio brasiliensis and Transcriptome Analysis. foods-11-00840.pdf

24. Thomas Brauge, Julia Mougin, Timothy Ells, Graziella Midelet (2023). Sources and contamination routes of seafood with human pathogenic Vibrio spp.: A Farm-to-Fork approach, 1-2 1541-4337.13283

25. Tody C.Lorenz.(2012). Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and OptimizationStrategies. https://pmc.ncbi.nlm.nih.gov/articles/PMC4846334/pdf/jove-63-3998.df

26. V. N Lydick, S. Mass, R. Peptin, R. Podicheti, E.Klempic, D. B Rusch, B. Ushijima, L. C Brown, D. Salomon, J. Kessel, ( Jan 2025). Quorum sensing regulates virulence factors in the coral pathogen *Vibrio coralliilyticus.* https://pmc.ncbi.nlm.nih.gov/articles/PMC11837519/

27. Xue Li, Junfang Sun, Miaomiao Zhang, Xingfan Xue, Qimin Wu, Wenhui Yang, Zhe Yin, Dongsheng Zhou, Renfei Lu,Yiquan Zhang,(2021).The Effect of Salinity on Biofilm Formation and C-di-GMP Production in Vibrio parahaemolytics. https://link.springer.com/article/10.1007/s00284-021-02723-2

28. Yi You Wong, Choon Weng Lee, Chui Wei Bong, Joon Hai Lim, Kumaran Narayanan, Edmund Ui Hang Sim, (September, 2019. Environmental Control of *Vibrio* spp. abundance and community structure in tropical waters. https://doi.org/10.1093/femsec/fiz176

29. Y. Ping, H.Zou, J. Li, T.Song, W. Lv, W. Wang, Z.Wang, S.Toa, (2021). Impact of quorum sensing signaling molecule in gram-negative bacteria on host cells: current understanding and future perspectives, https://pmc.ncbi.nlm.nih.gov/articles/PMC8865250/pdf/KGMI_14_2039048.pdf

30. Zhu, J., & Winans, S. C, (2001). The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization. Proceedings of the National Academy of Sciences, 98(4), 1507–1512.