Progress in Microbes and Molecular Biology

HH PUBLISHER

Genome Report

Complete Whole Genome Sequence of *Vibrio parahaemolyticus* RP0132 Strain Isolated from Shrimp in Malaysia

Ke-Yan Loo[1,7], Loh Teng-Hern Tan[1,2], Jodi Woan-Fei Law[1,3], Kah-Ooi Chua[4], Priyia Pusparajah[5], Learn-Han Lee[1,6*], Vengadesh Letchumanan[1,7*]

Article History

Received: 13 October 2023;

Received in Revised Form: 21 November 2023;

Accepted: 03 December 2023;

Available Online: 07 December 2023

[1]Novel Bacteria and Drug Discovery Research Group (NBDD), Microbiome and Bioresource Research Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway 47500, Selangor Darul Ehsan, Malaysia; ke.loo@monash.edu (K-YL)

[2]Innovative Bioprospection Development Research Group (InBioD), Clinical School Johor Bahru, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Johor Bahru 80100, Malaysia; loh.teng.hern@monash.edu (LT-HT)

[3]Next-Generation Precision Medicine and Therapeutics Research Group (NMeT), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway 47500, Selangor Darul Ehsan, Malaysia; jodi.law1@monash.edu (JW-FL)

[4]Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, Kuala Lumpur 50603, Malaysia; kahooi@um.edu.my (K-OC)

[5]Medical Health and Translational Research Group (MHTR), Microbiome and Bioresource Research, Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway, 47500, Selangor Darul Ehsan, Malaysia; priyia.pusparajah@monash.edu (PP)

[6]Sunway Microbiome Centre, School of Medical and Life Sciences, Sunway University, Sunway City 47500, Malaysia

[7]Pathogen Resistome Virulom and Diagnostic Research Group (PathRiD), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway 47500, Selangor Darul Ehsan, Malaysia

*Corresponding author: Vengadesh Letchumanan; Pathogen Resistome Virulome and Diagnostic Research Group (PathRiD), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway 47500, Selangor, Malaysia; vengadesh.letchumanan1@monash.edu (VL); Learn-Han Lee; Sunway Microbiome Centre, School of Medical and Life Sciences, Sunway University, Sunway City 47500, Malaysia; learnhanl@sunway.edu (L-HL)

Abstract: *Vibrio parahaemolyticus* is a Gram-negative, halophilic bacterium that is ubiquitous in marine environments. Its perilous co-existence with aquatic animals increases the risk of infections and diseases, especially those in aquaculture systems, thus resulting in reduced production and economic losses in the aquaculture industry. Moreover, *V. parahaemolyticus* can be easily transmitted to humans via consumption of contaminated seafood, resulting in gastroenteritis outbreaks. However, the rise in multidrug resistance

within the species has challenged the efficacy of antimicrobial treatments against *V. parahaemolyticus* infections. Therefore, we report the genome sequence of *V. parahaemolyticus* RP0132 isolated from shrimp to gain insight into its antimicrobial resistance traits and potential resistance mechanisms. The findings will facilitate the development of effective anti-*Vibrio* agents to manage *V. parahaemolyticus* infections.

Keywords: *Vibrio parahaemolyticus*; multi-drug resistance; genome; antibiotic; next generation sequencing

1. Introduction

Vibrio parahaemolyticus is a Gram-negative, halophilic bacterium that is abundant in aquatic environments such as estuaries, rivers, and oceans [1-3]. The cohabitation of this pathogen and marine animals increases the risk of infections and diseases, particularly in the cultivated animals in aquaculture systems [4-6]. This reduces seafood yield and causes massive economic losses within the aquaculture industry. Furthermore, *V. parahaemolyticus* can be easily transmitted to humans by consumption of contaminated seafood, resulting in gastroenteritis [7-11]. This seafood-borne pathogen has been associated with gastroenteritis outbreaks across various countries worldwide [12-14]. Treatment of *V. parahaemolyticus* typically focuses on rehydration and antimicrobial therapy [15], but the emergence of antibiotic resistance within the species has reduced the efficacy of the antibiotics [16]. The rise in antibiotic resistance (AMR) in *V. parahaemolyticus* can be attributed to the uncontrolled use of antibiotics in aquaculture, where antimicrobial agents are used as prophylaxis and treatment for infected cultivated animals [17-20]. Antibiotic residues from the aquaculture systems create environmental pressures that result in the development of AMR in *V. parahaemolyticus* isolates [21]. Multiple studies have also reported on multidrug resistant (MDR) *V. parahaemolyticus* strains [22-27]. Moreover, AMR can be spread inter- or intra-species via horizontal gene transfer, producing new generations of MDR bacteria in our surrounding environment [28-30]. Consequently, bacterial infections by MDR bacteria will become more prevalent, thereby increasing the difficulty of providing effective treatment, resulting in increased fatality rates [31, 32]. As an alternative to antibiotics, researchers have been exploring the use of probiotics as a means of preventing bacterial infections via gut microbiome modulation [33-35]. However, additional clinical research is still needed to produce conclusive evidence on the effectiveness of probiotics [34, 36, 37]. Therefore, it is crucial to maintain vigilant, ongoing surveillance of the AMR patterns of *V. parahaemolyticus* strains in our surrounding environment [38, 39]. These efforts are vital for safeguarding the aquaculture industry's viability and preserving public health.

With the increasing availability and accessibility of next-generation sequencing technologies [40, 41], whole genome analysis has provided extensive genomic information on various organisms [42-45]. To better understand the MDR patterns of *V. parahaemolyticus*, we examined the whole genome sequence (WGS) of *V. parahaemolyticus* RP0132, a strain obtained from our previous study [46]. The strain, RP0132 was isolated from speckled shrimp

(*Metapenaeus monoceros*), which originated from a wet market in Malaysia. This strain was resistant to five out of fourteen types of antibiotics tested with a multiple antibiotic resistance (MAR) index of 0.36. The antibiogram of RP0132 showed resistance towards ampicillin, third-generation cephalosporins (ceftazidime and cefotaxime), gentamicin, and the combination antibiotic, ampicillin/sulbactam. The AMR patterns of RP0132 raise concern as they depict resistance against the recommended antimicrobial agents in *V. parahaemolyticus* infections, such as third-generation cephalosporins and aminoglycosides [47, 48]. As AMR spreads, the antibiotic choice will become very limited when treating these bacterial infections. Their AMR and corresponding genes can be identified by examining the whole genome sequence of *V. parahaemolyticus* isolates [49, 50]. This can potentially help develop effective treatment for MDR *V. parahaemolyticus*. Therefore, the whole genome sequence of the MDR *V. parahaemolyticus* RP0132 strain was studied to gain insight into the potential mechanisms driving its resistance and enhance the understanding of AMR within the species.

2. Data description

The genomic DNA of RP0132 was extracted using MasterPure Complete DNA and RNA Purification Kit (LGC Biosearch Technologies) according to the manufacturer's instructions with slight modifications. The DNA quality and quantity were checked using agarose gel electrophoresis and Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). SMRTbell DNA libraries were generated according to standard protocols and checked with Qubit for quantification and bioanalyzer for size distribution detection. The whole genome of RP0132 was sequenced on PacBio Sequel II/IIe systems, yielding a genome coverage of 102.3-fold. After sequencing, the raw reads were assembled using Falcon which is based on the hierarchical genome assembly process (HGAP). BUSCO assessment was also done to assess the genome assembly and annotation completeness with single-copy orthologs. Ribosome RNA (rRNA) genes were analyzed by the RNAmmer and transfer RNA (tRNA) genes were predicted by the tRNAscan-SE. Annotation was performed using PATRIC (PathoSystems Resources Integration Center) for antibiotic-resistant genes. Further, the PATRIC-annotated antibiotic genes were BLAST against the National Center for Biotechnology Information (NCBI) database to confirm the gene identities.

The whole genome of RP0132 comprised of 2 contigs, and the assembled complete genome size of *V. parahaemolyticus* RP0132 contains 5,165,100 bp, with an average genome coverage of 102.3-fold with a GC content of 45%. The BUSCO score of the whole genome of RP0132 was 100%, indicating a reliable data output [51]. A total of 132 tRNA genes and 37 rRNA genes were predicted in the RP0132 genome (Table 1).

Table 1. Genomic features of *Vibrio parahaemolyticus* RP0132.

	Vibrio parahaemolyticus RP0132
Total number of contigs	2
Genome size (bp)	5,165,100
GC content (%)	45
Genome coverage	102.3x
DNA scaffold	2
Number of chromosomes	2
Total number of predicted genes	4797
Total number of protein-coding genes	4523
Total number of RNA-coding genes	165 (tRNA-coding-genes: 132, rRNA-coding genes: 33)
Total number of NCRNA-coding genes	4
Total number of pseudogenes	105

From the WGS of *V. parahaemolyticus* RP0132, multiple antibiotic resistance genes (ARG) were detected, and the findings corroborated the phenotype of the strain. For instance, the gene encoding for the detected aminoglycoside phosphotransferase is associated with the antibiotic resistance of RP0132 towards gentamicin, and the strain exhibited intermediate resistance towards kanamycin. Tian et al. identified a possible mechanistic explanation for the resistance towards aminoglycoside in *V. parahaemolyticus* whereby there is an upregulation of antibiotic resistance genes involved in phosphotransferase systems such as aminoglycoside phosphotransferases [52]. In addition, resistance towards the beta-lactams, including ampicillin, ampicillin/sulbactam, ceftazidime, and cefotaxime is closely related to the presence of ARGs encoding for metallo-beta-lactamase and carbenicillin-hydrolyzing class A beta-lactamase. Metallo beta-lactamases are enzymes that catalyze the hydrolysis of beta-lactam antibiotics by binding the negatively charged carboxylate or similarly charged group on beta-lactams with hydrogen bonds [53, 54]. Subsequent reactions involve the acylation of the beta-lactamase and deacylation of the beta-lactam-beta-lactamase complex with a water molecule. This will result in the inactivation of the antibiotic and the regeneration of an active beta-lactamase [55]. Currently, no metallo-bata-lactamase inhibitors are available, making resistance conferred via metallo-beta-lactamases a difficult challenge to overcome [56]. A study by Coutinho et al. determined the presence of CARB-18 gene, which encodes for a beta-lactamase in the studied *V. parahaemolyticus* strain JPA1 [57]. The presence of the beta lactamase was associated with the strain's resistance towards the beta-lactams such as ampicillin, ampicillin/sulbactam, and ceftazidime [57]. These findings are similar to those from the phenotype and genome of RP0132.

Besides, the presence of ARGs encoding for chloramphenicol acetyltransferase (CAT) conferred its intermediate resistance towards chloramphenicol. CAT is the most encountered resistance mechanism of bacteria towards chloramphenicol. The CAT enzymes inactivate the drug via acetylation to ensure bacteria survival [58, 59]. ARGs encoding for CAT have been found in *V. parahaemolyticus* and members of the *Vibrio* family, such as *V. cholerae* and *V. vulnificus* are known to cause human diseases [57, 60]. Moreover, multiple genes encoding for efflux pumps were also found within the genome, indicating other possible mechanisms of drug

efflux contributing to its antibiotic resistance (Table 2). These efflux pumps are active solute transport systems that pump the antibiotics into the extracellular space, allowing *V. parahaemolyticus* isolates to survive and proliferate even under highly distinct antimicrobial agents [61]. The ARGs in the genome of RP0132 encode efflux pumps that can be further categorized into resistance-nodulation-cell division (RND) and multidrug and toxic compound extrusion (MATE) families of efflux pumps. The expression of these efflux pumps can confer resistance towards aminoglycosides, beta-lactams, chloramphenicol, fluoroquinolones, novobiocin, rifampin, erythromycin, tetracyclines, and trimethoprim [62-64].

Table 2. Antibiotic resistance genes and their corresponding proteins detected from WGS of *V. parahaemolyticus* RP0132.

Antibiotic resistance genes	**BLAST result**
vmeA	Multidrug efflux RND transporter periplasmic adaptor subunit VmeA
vmeC	Multidrug efflux RND transporter periplasmic adaptor subunit VmeC
vmeE	Multidrug efflux RND transporter periplasmic adaptor subunit VmeE
vmeJ	Multidrug efflux RND transporter periplasmic adaptor subunit VmeJ
vmeT	Multidrug efflux RND transporter periplasmic adaptor subunit VmeT
vmeU	Multidrug efflux RND transporter periplasmic adaptor subunit VmeU
vmeY	Multidrug efflux RND transporter periplasmic adaptor subunit VmeY
vmeB	Multidrug efflux RND transporter permease subunit VmeB
vmeD	Multidrug efflux RND transporter permease subunit VmeD
vmeF	Multidrug efflux RND transporter permease subunit VmeF
vmeK	multidrug efflux RND transporter permease subunit VmeK
vmeV	multidrug efflux RND transporter permease subunit VmeV
vmeZ	multidrug efflux RND transporter permease subunit VmeZ
vmeI	efflux RND transporter permease subunit VmeI
vmeQ	efflux RND transporter permease subunit VmeQ
vmeG	efflux RND transporter periplasmic adaptor subunit VmeG
vmeH	efflux RND transporter periplasmic adaptor subunit VmeH
abgT	AbgT family transporter
bcr/*cflA*	Bcr/CflA family multidrug efflux MFS transporter
dinF	MATE family efflux transporter DinF

The MDR patterns of *V. parahaemolyticus* RP0132 observed through both phenotype and genotype provide insight into the extent of antibiotic contamination in the environment. The MAR index of RP0132 is greater than 0.2, indicating the strain originated from a high-risk source of contamination where antibiotics are frequently used [65, 66]. This could be attributed to the extensive use of antibiotics in aquaculture, which remains a major contributor to AMR in *V. parahaemolyticus* [67]. Moreover, the effects of climate change, such as the increase in seawater temperatures, have driven the growth of *V. parahaemolyticus* populations in the environment [13, 68]. With the rise in global temperatures, infectious diseases are becoming

more apparent, jeopardizing the livelihoods of global citizens [68-70]. Therefore, there is a dire need to search for solutions to resolve this public health issue to protect the public from AMR infections. Recent research has been looking into alternative treatment methods to manage the populations of *V. parahaemolyticus* and reduce the spread of AMR in our surrounding environments [71-74]. A compelling candidate is *Streptomyces* sp., a group of Gram-positive, filamentous bacteria belonging to the phylum *Actinobacteria* [75]. They are abundant in nature and have been frequently isolated from various sources such as soils, mangroves, and marine environments [76-81]. The use of streptomycetes as a probiotic has been widely studied due to their ability to produce bioactive secondary metabolites during their life cycle [82-88]. Moreover, *Streptomyces* sp. has shown various bioactivities, including antioxidative, anticancer, cytotoxic, antibacterial, and more specifically anti-*Vibrio* properties [46, 89-91].

Research has shown the various potential mechanisms behind the anti-*Vibrio* properties of *Streptomyces* sp [92-95]. For instance, the production of siderophores or enzymes by streptomycetes has been shown to inhibit the growth of *Vibrios* in aquatic environments [96, 97]. In addition, the production of melanin compounds from marine *Streptomyces* has exhibited good activity against *Vibrios* including *V. parahaemolyticus* [98-100]. Furthermore, the supplementation of *Streptomyces* sp. in aquatic animals has increased the population of antimicrobial producers in the host, thereby increasing the host immunity and defense against *Vibrios* [101, 102]. Moreover, the supplementation of *Streptomyces* sp. in marine animals stimulated the release of growth hormones in the host, thereby increasing the growth rates of the marine animals [103, 104]. Hence, using *Streptomyces* as a probiotic can help control *Vibrio* populations while simultaneously increasing host immunity against *Vibrios* and increasing the growth rates of the cultivated animals. However, the developmental process of new antibiotics or alternatives is rigorous and time-consuming, and the rapid emergence of AMR may outpace the innovation timeline [105-108]. Therefore, it is crucial to establish and implement stringent regulations and policies governing the use of antibiotics in aquaculture systems [109, 110]. This is vital in safeguarding and preserving antibiotics' efficacy, ultimately upholding food safety and public health. In summary, the whole genome sequence of *V. parahaemolyticus* RP0132 provides a better understanding of the AMR patterns and the underlying resistance mechanisms within *V. parahaemolyticus*.

The whole genome sequence of *Vibrio parahaemolyticus* R0132 has been deposited at DDBJ/EMBL/GenBank under accession numbers CP131930.1 and CP131931.1. The version described in this genome report is the first version. The genome data is publicly available at NCBI GenBank under the BioProject accession number PRJNA1000768, and the BioSample accession number SAMN36780134.

Author Contributions: K-YL conducted the laboratory research work, data analysis and manuscript writing. K-OC provided support on the data analysis and proofreading. LT-HT, JW-FL, PP, L-HL and VL provided resources, supervision, proofreading and technical support. VL and L-HL conceptualized and founded this writing project. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by the Jeffrey Cheah School of Medicine and Health Sciences Strategic Grant 2021 (Vote Number: STG-000051) awarded to VL and L-HL.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Letchumanan V, Chan K-G, and Lee L-H. Vibrio parahaemolyticus: a review on the pathogenesis, prevalence, and advance molecular identification techniques. Front Microbiol 2014; 5: 705.
2. Venggadasamy V, Tan LT-H, Law JW-F, *et al*. Incidence, antibiotic susceptibility and characterization of Vibrio parahaemolyticus isolated from seafood in selangor, Malaysia. Prog Microbes Mol Bio 2021; 4(1).
3. Frieri M, Kumar K, and Boutin A. Antibiotic resistance. J Infect Public Health 2017; 10(4): 369-378.
4. Mok JS, Cho SR, Park YJ, *et al*. Distribution and antimicrobial resistance of Vibrio parahaemolyticus isolated from fish and shrimp aquaculture farms along the Korean coast. Mar Pollut Bull 2021; 171: 112785.
5. Novriadi R. Vibriosis in aquaculture. Omni-Akuatika 2016; 12(1).
6. Sony M, Sumithra T, Anusree V, *et al*. Antimicrobial resistance and virulence characteristics of Vibrio vulnificus, Vibrio parahaemolyticus and Vibrio harveyi from natural disease outbreaks of marine/estuarine fishes. Aquaculture 2021; 539: 736608.
7. Loo K-Y, Law JW-F, Tan LT-H, *et al*. The burden of Vibrio sp. infections–A scoping review. Prog Microbes Mol Bio 2023; 6(1).
8. Letchumanan V, Loo K-Y, Law JW-F, *et al*. Vibrio parahaemolyticus: The protagonist of foodborne diseases. Prog Microbes Mol Bio 2019; 2(1).
9. Guin S, Saravanan M, Chowdhury G, *et al*. Pathogenic Vibrio parahaemolyticus indiarrhoeal patients, fish and aquatic environments and their potential for inter-source transmission. Heliyon 2019; 5(5).
10. Letchumanan V, Pusparajah P, Tan LT-H, *et al*. Occurrence and antibiotic resistance of Vibrio parahaemolyticus from shellfish in Selangor, Malaysia. Front Microbiol 2015; 6: 1417.
11. Lee L-H, Loo K-Y, Tan LT-H, *et al*. *IDDF2021-ABS-0126 Exploring the gut microbiota variation in response to vibrio infection*. 2021, BMJ Publishing Group.
12. Park K, Mok JS, Kwon JY, *et al*. Food-borne outbreaks, distributions, virulence, and antibiotic resistance profiles of Vibrio parahaemolyticus in Korea from 2003 to 2016: a review. Fish Aquatic Sci 2018; 21: 1-10.
13. Hsiao H-I, Jan M-S, and Chi H-J. Impacts of Climatic Variability on Vibrio parahaemolyticus Outbreaks in Taiwan. Int J Environ Res Public Health 2016; 13(2): 188.
14. Bonnin-Jusserand M, Copin S, Le Bris C, *et al*. Vibrio species involved in seafood-borne outbreaks (Vibrio cholerae, V. parahaemolyticus and V. vulnificus): Review of microbiological versus recent molecular detection methods in seafood products. Crit Rev Food Sci Nutr 2019; 59(4): 597-610.
15. Wang R, Zhong Y, Gu X, *et al*. The pathogenesis, detection, and prevention of Vibrio parahaemolyticus. Front Microbiol 2015; 6: 144.
16. Letchumanan V, Ab Mutalib N-S, Wong SH, *et al*. Determination of antibiotic resistance patterns of Vibrio parahaemolyticus from shrimp and shellfish in Selangor, Malaysia. Prog Microbes Mol Bio 2019; 2(1).
17. Hossain A, Habibullah-Al-Mamun M, Nagano I, *et al*. Antibiotics, antibiotic-resistant bacteria, and resistance genes in aquaculture: risks, current concern, and future thinking. Environ Sci Pollut Res 2022; 29(8): 11054-11075.
18. Xiong W, Sun Y, Zhang T, *et al*. Antibiotics, Antibiotic Resistance Genes, and Bacterial Community Composition in Fresh Water Aquaculture Environment in China. Microb Ecol 2015; 70(2): 425-432.

19. Shah SQ, Cabello FC, L'Abée-Lund TM, *et al*. Antimicrobial resistance and antimicrobial resistance genes in marine bacteria from salmon aquaculture and non-aquaculture sites. Environ Microbiol 2014; 16(5): 1310-1320.
20. Romero J, Feijoó CG, and Navarrete P. *Antibiotics in aquaculture–use, abuse and alternatives*, in *Health and environment in aquaculture*. 2012. p. 159-198.
21. Preena PG, Swaminathan TR, Kumar VJR, *et al*. Antimicrobial resistance in aquaculture: a crisis for concern. Biologia 2020; 75: 1497-1517.
22. Igbinosa EO. Detection and antimicrobial resistance of Vibrio isolates in aquaculture environments: implications for public health. Microb Drug Resist 2016; 22(3): 238-245.
23. Elmahdi S, DaSilva LV, and Parveen S. Antibiotic resistance of Vibrio parahaemolyticus and Vibrio vulnificus in various countries: a review. Food Microbiol 2016; 57: 128-134.
24. Dutta D, Kaushik A, Kumar D, *et al*. Foodborne pathogenic vibrios: antimicrobial resistance. Front Microbiol 2021; 12: 638331.
25. Lee L-H, Ab Mutalib N-S, Law JW-F, *et al*. Discovery on antibiotic resistance patterns of Vibrio parahaemolyticus in Selangor reveals carbapenemase producing Vibrio parahaemolyticus in marine and freshwater fish. Front Microbiol 2018; 9: 2513.
26. Goh JXH, Tan LT-H, Law JW-F, *et al*. Insights into Carbapenem Resistance in Vibrio Species: Current Status and Future Perspectives. International Journal of Molecular Sciences 2022; 23(20): 12486.
27. Fang J, Cheng H, Yu T, *et al*. Occurrence of virulence factors and antibiotic and heavy metal resistance in Vibrio parahaemolyticus isolated from pacific mackerel at markets in Zhejiang, China. J Food Prot 2020; 83(8): 1411-1419.
28. Loo KY, Letchumanan V, Law JWF, *et al*. Incidence of antibiotic resistance in Vibrio spp. Rev Aquac 2020; 12(4): 2590-2608.
29. Larsson DJ and Flach C-F. Antibiotic resistance in the environment. Nat Rev Microbiol 2022; 20(5): 257-269.
30. Ong IJ, Loo K-Y, Law LN-S, *et al*. Exploring the impact of Helicobacter pylori and potential gut microbiome modulation. Prog Microbes Mol Bio 2023; 6(1): a0000273.
31. Kumar SB, Arnipalli SR, and Ziouzenkova O. Antibiotics in Food Chain: The Consequences for Antibiotic Resistance. Antibiotics 2020; 9(10): 688.
32. French GL. The continuing crisis in antibiotic resistance. Int J Antimicrob Agents 2010; 36: S3-S7.
33. Sim AAXH, Cheam JY, Law JW-F, *et al*. The ameliorative role of probiotics in 5-fluorouracil induced intestinal mucositis. Prog Microbes Mol Bio 2023; 6(1).
34. Lau AWY, Tan LT-H, Ab Mutalib N-S, *et al*. The chemistry of gut microbiome in health and diseases. Prog Microbes Mol Bio 2021; 4(1).
35. Defoirdt T, Sorgeloos P, and Bossier P. Alternatives to antibiotics for the control of bacterial disease in aquaculture. Curr Opin Microbiol 2011; 14(3): 251-258.
36. Selvaraj SM, Wong SH, Ser H-L, *et al*. Role of low FODMAP diet and probiotics on gut microbiome in irritable bowel syndrome (IBS). Prog Microbes Mol Bio 2020; 3(1).
37. Rostami FM, Mousavi H, Mousavi MRN, *et al*. Efficacy of Probiotics in Prevention and Treatment of Infectious Diseases. Clin Microbiol Newsl 2018; 40(12): 97-103.
38. Loo K-Y, Law JW-F, Tan LT-H, *et al*. Diagnostic techniques for rapid detection of Vibrio species. Aquaculture 2022: 738628.

39. Loo K-Y, Law JW-F, Tan LT-H, *et al*. IDDF2022-ABS-0201 Vibrio: detecting you earlier, avoiding gastroenteritis and GUT microbiome dysbiosis. Gut 2022; 71(Suppl 2): A53-A54.
40. Satam H, Joshi K, Mangrolia U, *et al*. Next-generation sequencing technology: Current trends and advancements. Biology 2023; 12(7): 997.
41. Giani AM, Gallo GR, Gianfranceschi L, *et al*. Long walk to genomics: History and current approaches to genome sequencing and assembly. Comput Struct Biotechnol J 2020; 18: 9-19.
42. Goh YX, Chan KG, and Hong KW. Whole-genome sequence of Chelatococcus daeguensis strain M38T9, Isolated from Ulu Slim hot spring in Malaysia. Prog Microbes Mol Bio 2022; 5(1).
43. Ser H-L, Tan W-S, Yin W-F, *et al*. Whole genome sequence of MUM116, a Bacillus species isolated from intertidal soil. Prog Microbes Mol Bio 2020; 3(1).
44. Letchumanan V, Ser H-L, Chan K-G, *et al*. Genome sequence of Vibrio parahaemolyticus VP103 strain isolated from shrimp in Malaysia. Front Microbiol 2016; 7: 1496.
45. Letchumanan V, Ser H-L, Tan W-S, *et al*. Genome sequence of Vibrio parahaemolyticus VP152 strain isolated from Penaeus indicus in Malaysia. Front Microbiol 2016; 7: 1410.
46. Loo K-Y, Tan LT-H, Law JW-F, *et al*. Detection of multidrug resistant Vibrio parahaemolyticus and anti-Vibrio Streptomyces sp. MUM 178J. Prog Microbes Mol Bio 2023; 6(1).
47. Shaw KS, Rosenberg Goldstein RE, He X, *et al*. Antimicrobial Susceptibility of Vibrio vulnificus and Vibrio parahaemolyticus Recovered from Recreational and Commercial Areas of Chesapeake Bay and Maryland Coastal Bays. PLOS ONE 2014; 9(2): e89616.
48. Lopatek M, Wieczorek K, and Osek J. Prevalence and Antimicrobial Resistance of Vibrio parahaemolyticus Isolated from Raw Shellfish in Poland. J Food Prot 2015; 78(5): 1029-1033.
49. Letchumanan V, Tan W-S, Yin W-F, *et al*. Genome sequence of Vibrio sp. OULL4 isolated from shellfish. Prog Microbes Mol Bio 2020; 3(1).
50. Letchumanan V, Ser H-L, Tan W-S, *et al*. Genome sequence of Vibrio sp. SALL 6 isolated from shellfish. Prog Microbes Mol Bio 2019; 2(1).
51. Feron R and Waterhouse RM. Assessing species coverage and assembly quality of rapidly accumulating sequenced genomes. Gigascience 2022; 11.
52. Tian C, Yuan M, Tao Q, *et al*. Discovery of Novel Resistance Mechanisms of Vibrio parahaemolyticus Biofilm against Aminoglycoside Antibiotics. Antibiotics 2023; 12(4): 638.
53. Wang Z, Fast W, Valentine AM, *et al*. Metallo-β-lactamase: structure and mechanism. Curr Opin Chem Biol 1999; 3(5): 614-622.
54. Palzkill T. Metallo-β-lactamase structure and function. Ann N Y Acad Sci 2013; 1277(1): 91-104.
55. Bush K and Bradford PA. Interplay between β-lactamases and new β-lactamase inhibitors. Nat Rev Microbiol 2019; 17(5): 295-306.
56. Mojica MF, Rossi M-A, Vila AJ, *et al*. The urgent need for metallo-β-lactamase inhibitors: an unattended global threat. Lancet Infect Dis 2022; 22(1): e28-e34.
57. Coutinho FH, Tschoeke DA, Clementino MM, *et al*. Genomic basis of antibiotic resistance in Vibrio parahaemolyticus strain JPA1. Mem Inst Oswaldo Cruz 2019; 114: e190053.
58. Schwarz S, Kehrenberg C, Doublet B, *et al*. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev 2004; 28(5): 519-542.
59. Shaw WV. Chloramphenicol acetyltransferase: enzymology and molecular biology. Crit Rev Biochem 1983; 14(1): 1-46.

60. Alcala A, Ramirez G, Solis A, *et al*. Structural and functional characterization of three Type B and C chloramphenicol acetyltransferases from Vibrio species. Protein Sci 2020; 29(3): 695-710.
61. Blair JM, Webber MA, Baylay AJ, *et al*. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 2015; 13(1): 42-51.
62. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol 2018; 4(3): 482-501.
63. Kumar S, Floyd JT, He G, *et al*. Bacterial antimicrobial efflux pumps of the MFS and MATE transporter families: a review. Recent Res Dev Antimicrob Agents Chemother 2013; 7: 1-21.
64. Murakami S. *Structures and transport mechanisms of RND efflux pumps*, in *Efflux-mediated antimicrobial resistance in bacteria: Mechanisms, regulation and clinical implications*. 2016. p. 3-28.
65. Davis R and Brown PD. Multiple antibiotic resistance index, fitness and virulence potential in respiratory Pseudomonas aeruginosa from Jamaica. J Med Microbiol 2016; 65(4): 261-271.
66. Elhadi N, Yamani LZ, Aljeldah M, *et al*. Serological and Antibiotic Resistance Patterns As Well As Molecular Characterization of Vibrio parahaemolyticus Isolated from Coastal Waters in the Eastern Province of Saudi Arabia. J Epidemiol Glob Health 2022; 12(4): 524-540.
67. Chen J, Sun R, Pan C, *et al*. Antibiotics and food safety in aquaculture. J Agric Food Chem 2020; 68(43): 11908-11919.
68. Froelich BA and Daines DA. In hot water: effects of climate change on Vibrio–human interactions. Environ Microbiol 2020; 22(10): 4101-4111.
69. Baker-Austin C, Stockley L, Rangdale R, *et al*. Environmental occurrence and clinical impact of Vibrio vulnificus and Vibrio parahaemolyticus: a European perspective. Environ Microbiol Rep 2010; 2(1): 7-18.
70. Baker RE, Mahmud AS, Miller IF, *et al*. Infectious disease in an era of global change. Nat Rev Microbiol 2022; 20(4): 193-205.
71. Goh JXH, Tan LT-H, Law JW-F, *et al*. Probiotics: Comprehensive Exploration of the Growth Promotion Mechanisms in Shrimps. Prog Microbes Mol Bio 2023; 6(1).
72. Battah B, Rajab A, Shbibe L, *et al*. Evaluation of antibiofilm activity of Thymus syriacus essential oil against clinically isolated MDR bacteria. Prog Microbes Mol Bio 2022; 5(1).
73. Ong K-S, Letchumanan V, Law JW-F, *et al*. Microbes from Peat Swamp Forest—The Hidden Reservoir for Secondary Metabolites? Prog Microbes Mol Bio 2020; 3(1).
74. Tan W-S, Law JW-F, Law LN-S, *et al*. Insights into quorum sensing (QS): QS-regulated biofilm and inhibitors. Prog Microbes Mol Bio 2020; 3(1).
75. Law JWF, Tan LT-H, Letchumanan V, *et al*. Streptomyces griseiviridis sp. nov., a Novel "Modern Actinobacteria" isolated from Malaysia Mangrove Soil. Prog Microbes Mol Bio 2023; 6(1).
76. Lee L-H, Letchumanan V, Ab Mutalib N-S, *et al*. Microbial community diversity in the soil of Barrientos Island estimated by RAPD and Biolog Ecoplate methods. Prog Microbes Mol Bio 2020; 3(1).
77. Dharmaraj S. Marine Streptomyces as a novel source of bioactive substances. World J Microbiol Biotechnol 2010; 26: 2123-2139.
78. Al-Ansari M, Alkubaisi N, Vijayaragavan P, *et al*. Antimicrobial potential of Streptomyces sp. to the Gram positive and Gram negative pathogens. J Infect Public Health 2019; 12(6): 861-866.

79. Al-Dhabi NA, Esmail GA, Ghilan A-KM, *et al*. Isolation and purification of starch hydrolysing amylase from Streptomyces sp. Al-Dhabi-46 obtained from the Jazan region of Saudi Arabia with industrial applications. J King Saud Univ Sci 2020; 32(1): 1226-1232.
80. Jaroszewicz W, Bielańska P, Lubomska D, *et al*. Antibacterial, antifungal and anticancer activities of compounds produced by newly isolated Streptomyces strains from the Szczelina Chochołowska cave (Tatra Mountains, Poland). Antibiotics 2021; 10(10): 1212.
81. Al Farraj DA, Varghese R, Vágvölgyi C, *et al*. Antibiotics production in optimized culture condition using low cost substrates from Streptomyces sp. AS4 isolated from mangrove soil sediment. J King Saud Univ Sci 2020; 32(2): 1528-1535.
82. Pusparajah P, Law JW-F, Chan K-G, *et al*. Whole-Genome Sequence of Streptomyces pluripotens strain MUM 16J, a Potential Resource of Glycopeptide Antibiotic and Biocontrol Agent against Biofilm-forming Bacteria. Prog Microbes Mol Bio 2023; 6(1).
83. Thye AY-K, Letchumanan V, Tan LT-H, *et al*. Malaysia's Breakthrough in Modern Actinobacteria (MOD-ACTINO) Drug Discovery Research. Prog Microbes Mol Bio 2022; 5(1).
84. Elsalam RM, Goh KW, Mahadi M, *et al*. The Antibacterial Activities of Secondary Metabolites Derived from Streptomyces sp. Prog Microbes Mol Bio 2022; 5(1).
85. Ser H-L, Tan LT-H, Tan W-S, *et al*. Whole-genome sequence of bioactive streptomycete derived from mangrove forest in Malaysia, Streptomyces sp. MUSC 14. Prog Microbes Mol Bio 2021; 4(1).
86. Kemung HM, Tan LT-H, Chan K-G, *et al*. Streptomyces sp. strain MUSC 5 from mangrove forest in Malaysia: Identification, antioxidant potential and chemical profiling of its methanolic extract. Prog Microbes Mol Bio 2020; 3(1).
87. Law JW-F, Letchumanan V, Tan LT-H, *et al*. The rising of "modern actinobacteria" era. Prog Microbes Mol Bio 2020; 3(1).
88. Ab Mutalib N-S, Wong SH, Ser H-L, *et al*. Bioprospecting of microbes for valuable compounds to mankind. Prog Microbes Mol Bio 2020; 3(1).
89. Law JW-F, Letchumanan V, Hong K-W, *et al*. Streptomyces learnhanii sp. nov., unveiling a Mangrove-Derived Novel "Modern Actinobacteria" in Malaysia. Prog Microbes Mol Bio 2023; 6(1).
90. Ser H-L, Law JW-F, Tan W-S, *et al*. Whole genome sequence of Streptomyces colonosanans strain MUSC 93JT isolated from mangrove forest in Malaysia. Prog Microbes Mol Bio 2020; 3(1).
91. Beroigui O and Errachidi F. Streptomyces at the Heart of Several Sectors to Support Practical and Sustainable Applications: A Review. Prog Microbes Mol Bio 2023; 6(1).
92. Loo K-Y, Tan LT-H, Law JW-F, *et al*. Vibrio parahaemolyticus: Exploring its Incidence in Malaysia and the Potential of Streptomyces sp. as an Anti-Vibrio Agent. Prog Microbes Mol Bio 2023; 6(1).
93. Tan LT-H, Lee L-H, and Goh B-H. Critical review of fermentation and extraction of anti-Vibrio compounds from Streptomyces. Prog Microbes Mol Bio 2020; 3(1).
94. Tan LT-H, Lee L-H, and Goh B-H. The bioprospecting of anti-Vibrio Streptomyces species: Prevalence and applications. Prog Microbes Mol Bio 2019; 2(1).
95. Bi Y, Liu G, Yu Q, *et al*. Anti-Vibrio dibutyl phthalate from marine-derived Streptomyces sp. S073. Res Vet Sci 2021; 140: 198-202.
96. Yang M, Zhang J, Liang Q, *et al*. Antagonistic activity of marine Streptomyces sp. S073 on pathogenic Vibrio parahaemolyticus. Fish Sci 2019; 85: 533-543.

97. Augustine D, Jacob JC, and Philip R. Exclusion of Vibrio spp. by an antagonistic marine actinomycete S treptomyces rubrolavendulae M56. Aquac Res 2016; 47(9): 2951-2960.
98. Sivaperumal P, Kamala K, and Rajaram R. Bioactive DOPA melanin isolated and characterised from a marine actinobacterium Streptomyces sp. MVCS6 from Versova coast. Nat Prod Res 2015; 29(22): 2117-2121.
99. Vasanthabharathi V, Lakshminarayanan R, and Jayalakshmi S. Melanin production from marine Streptomyces. Afr J Biotechnol 2011; 10(54): 11224.
100. Sivaperumal P, Kamala K, Rajaram R, *et al*. Melanin from marine Streptomyces sp. (MVCS13) with potential effect against ornamental fish pathogens of Carassius auratus (Linnaeus, 1758). Biocatal Agric Biotechnol 2014; 3(4): 134-141.
101. Mazón-Suástegui JM, Salas-Leiva JS, Medina-Marrero R, *et al*. Effect of Streptomyces probiotics on the gut microbiota of Litopenaeus vannamei challenged with Vibrio parahaemolyticus. Microbiologyopen 2020; 9(2): e967.
102. Crossman LC, Chen H, Cerdeno-Tárraga A-M, *et al*. A small predatory core genome in the divergent marine Bacteriovorax marinus SJ and the terrestrial Bdellovibrio bacteriovorus. ISME J 2013; 7(1): 148-160.
103. Dharmaraj S and Dhevendaran K. Evaluation of Streptomyces as a probiotic feed for the growth of ornamental fish Xiphophorus helleri. Food Technol Biotechnol 2010; 48(4): 497-504.
104. Aftabuddin S, Kashem MA, Kader MA, *et al*. Use of Streptomyces fradiae and Bacillus megaterium as probiotics in the experimental culture of tiger shrimp Penaeus monodon (Crustacea, Penaeidae). Aquac Aquar Conserv Legis 2013; 6(3): 253-267.
105. Gajdács M and Albericio F. Antibiotic Resistance: From the Bench to Patients. Antibiotics 2019; 8(3): 129.
106. Miethke M, Pieroni M, Weber T, *et al*. Towards the sustainable discovery and development of new antibiotics. Nat Rev Chem 2021; 5(10): 726-749.
107. Hegemann JD, Birkelbach J, Walesch S, *et al*. Current developments in antibiotic discovery: Global microbial diversity as a source for evolutionary optimized anti-bacterials. EMBO Rep 2023; 24(1): e56184.
108. Cook MA and Wright GD. The past, present, and future of antibiotics. Sci Transl Med 2022; 14(657): eabo7793.
109. Bloomer E and McKee M. Policy options for reducing antibiotics and antibiotic-resistant genes in the environment. J Public Health Policy 2018; 39: 389-406.
110. Topp E, Larsson DJ, Miller DN, *et al*. Antimicrobial resistance and the environment: assessment of advances, gaps and recommendations for agriculture, aquaculture and pharmaceutical manufacturing. FEMS Microbiol Ecol 2018; 94(3): fix185.