Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Insights from whole-genome sequencing and comparative genomic analysis of the pathogenic Photobacterium damselae subsp. damselae strain MRY0520 isolated from Litopenaeus vannamei

Libao Wang
Institute of Oceanology & Marine Fisheries
Zhijun Yu
Beyotime Institute of Biotechnology
Qiuwen Xu
Tongzhou District Agriculture and Forestry Science and Education Management Station
Wenjun Shi
Institute of Oceanology & Marine Fisheries
Hui Li
Institute of Oceanology & Marine Fisheries
YingLi Yang
Institute of Oceanology & Marine Fisheries
Kuipeng Fu
Institute of Oceanology & Marine Fisheries
Xihe Wan
wxh1708@163.com

Institute of Oceanology & Marine Fisheries

Research Article

Keywords: Photobacterium damselae subsp. damselae, Whole genome sequencing, Genome map, Functional annotation, Comparative genomic analysis

Posted Date: March 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9004776/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Insights from whole-genome sequencing and comparative genomic analysis of the pathogenic *Photobacterium damselae* subsp. *damselae* strain MRY0520 isolated from *Litopenaeus vannamei*

Libao Wang[1] · Zhijun Yu[2] · Qiuwen Xu[3] · Wenjun Shi[1] · Hui Li[1] · YingLi Yang[1] ··Kuipeng Fu[1] · Xihe Wan[1]

[1]Institute of Oceanology & Marine Fisheries, Jiangsu, Nantong, China;

[2]Beyotime Institute of Biotechnology, Shanghai, China;

[3]Tongzhou District Agriculture and Forestry Science and Education Management Station, Jiangsu, Nantong, China;

*** Corresponding author:** Dr. Xihe Wan
Address: Institute of Oceanology & Marine Fisheries, Jiangsu, No. 31, Jiaoyu Road, Nantong, Jiangsu Province 226007, China
Tel: 86-0513-85228266
E-mail: wxh1708@163.com

Abstract

Photobacterium damselae is a widespread pathogenic bacterium in marine ecosystems, primarily associated with fish skin ulcers. However, infections caused by this bacterium in shrimp are rarely reported. The dominant strain MRY0520, isolated from the hepatopancreas of diseased *Litopenaeus vannamei*, was identified as *Photobacterium damselae* subsp. *damselae* (Phdd) and exhibited high pathogenicity. Elucidating the genetic architecture and infection mechanisms of *P. damselae* is crucial for the effective prevention and control of related diseases. To characterize its genomic features, whole-genome sequencing and comprehensive functional annotation were performed on the virulent Phdd strain MRY0520, followed by comparative genomic analysis to investigate its evolutionary traits. The genomic sequences have been deposited in GenBank under accession numbers CP113238–CP113240. The genome of strain MRY0520 spans 4,451,849 bp and contains 3,663 coding genes with a combined length of 3,691,779 bp, accounting for 83% of the total genome. It encodes 208 tRNA genes, 62 rRNA genes (including 22 5S rRNA, 20 16S rRNA, and 20 23S rRNA), two CRISPR arrays, and two genomic islands. Comparative analysis revealed that strain MRY0520 exhibits the highest genomic homology with Phdd strain Wu-1. These findings provide a solid theoretical basis for understanding the genomic composition and evolutionary characteristics of MRY0520.

Keywords *Photobacterium damselae* subsp. *damselae* · Whole genome sequencing · Genome map · Functional annotation · Comparative genomic analysis

Introduction

Photobacterium damselae, part of the Vibrionaceae family, consists of two distinct subspecies: subsp. *piscicida* and subsp. *damselae* (Chiu et al. 2013). The first subspecies was initially identified and isolated from *Morone americana* and *M. saxatilis* in 1963, leading to a disease originally termed Pasteurella, now known as luminescent bacterial disease (Thyssen et al. 1998). The second subspecies was first isolated from a skin ulcer on a diseased fish in 1981 and was subsequently named *Vibrio damsela* (Love et al. 1981). *P. damselae* subsp. *damselae* (Phdd) is a generalist heterotroph found widely in marine environments, including marine animals. It acts as an opportunistic pathogen, sometimes causing disease in a variety of hosts, including humans, with its virulence enhanced by a plasmid that expresses an additional copy of the major hemolysin. Conversely, *P. damselae* subsp. *piscicida* (Phdp) is a more specialized, possibly obligate, pathogen of marine fish. Although it shares a ribosome and approximately 80% of its genome with Phdd, it is highly specialized, possessing a significantly smaller genome and almost always carrying a small virulence plasmid pPHDP10, which includes a distinct AB apoptogenic toxin known as AIP56 (Baseggio et al. 2021). Recently, *P. damselae* subsp. *damselae* has been isolated from various diseased marine fish species, such as *Oncorhynchus mykiss*, *Dicentrarchus labrax*, and *Sebastes schlegeli* (Pedersen et al. 2009; Labella et al. 2010; Zhang et al. 2019). Additionally, this bacterium can infect mammals, including dolphins and humans (Rivas et al. 2013)

There have been very few instances of Phdd infecting shrimp. Two pathogenic strains of *Photobacterium* were isolated from *Exopalaemon carinicauda*, leading to shrimp mortality (Liu et al. 2016). Additionally, a *Photobacterium* strain was isolated and identified from *Penaeus monodon* and *Litopenaeus vannamei*, both suffering from "black gill" disease (Vaseeharan et al. 2007; Wang et al. 2020). In the initial phase, our laboratory identified a strain, MRY0520, as *P. damselae* from the hepatopancreas of *L. vannamei* affected by "Dixing disease" in Rudong County (Jiangsu Province). This strain was the causative agent of the disease and exhibited high pathogenicity (Wang et al. 2024). Diseased shrimp typically show symptoms of hepatopancreas atrophy and whitening, along with reduced vitality, empty stomachs, and intestines, resulting in significant economic losses in shrimp farming. To gain a further understanding of this strain's genetic background and evolutionary traits, we conducted whole-genome sequencing and functional annotation analysis of strain MRY0520, identified as Phdd. Additionally, a comparative genome analysis was performed between this strain and a model strain from the NCBI database to explore their phylogenetic relationships. This research provided new insights into this significant shrimp pathogen and its evolutionary characteristics.

Materials and methods

Collection of diseased *L. vannamei* and histopathological analysis

In Rudong County, Nantong, Jiangsu Province, diseased *L. vannamei* shrimp, measuring 6.45 ± 0.35 cm in length and weighing 8.5 ± 0.45 g, were gathered from a shrimp farm. A month after breeding, sporadic deaths began to occur, with some dead shrimp appearing daily on the feeding platform. The hepatopancreas (HPs) of the infected shrimp were removed and immediately placed in Bouin's fixative, which consists of 100 mL of 75% v/v saturated aqueous picric acid, 20 mL of 37% formaldehyde, and 5

mL of glacial acetic acid, for 24 h. They were then decolorized using 75% alcohol. Following dehydration and washing, the tissue was embedded in paraffin and sliced into thin sections. These paraffin sections were washed in xylene, rehydrated through an alcohol gradient, and stained with hematoxylin and eosin (H&E) as described by Cardiff et al. (2014). The prepared sections were then examined under a microscope to observe the HPs cells of the shrimp.

Isolation and identification of dominant culturable bacteria from diseased *L. vannamei*

The surface of diseased shrimp was alternately cleaned multiple times with sterile physiological saline and 75% alcohol. Subsequently, the HPs, gut, gills, and muscle tissues were sampled, and each was homogenized with sterilized 0.85% physiological saline. Tenfold serial dilutions of the homogenates were spread onto sterile Luria-Bertani (LB) agar plates (containing 10 g/L peptone, 5 g/L yeast extract, 30 g/L NaCl, and 20 g/L agar) and incubated at 25 °C for 24 h. Dominant single colonies were then selected for streak plate purification and inoculated into LB liquid medium for an additional 24 h of pure culture (shaking at 25 ℃, 180 rpm). Pure colonies were stored in 30% glycerol (v/v) at -80 ℃ for future use.

Injection challenge test with strain MRY0520

To further assess the pathogenicity of strain MRY0520, a controlled reinfection challenge experiment was conducted on strain MRY0520. Healthy *L. vannamei* (mean body length: 5.30 ± 0.30 cm; mean body weight: 3.58 ± 0.35 g) were obtained from the Rudong Experiment Base, Jiangsu Institute of Oceanology and Marine Fisheries. All shrimp exhibited robust vitality and normal hepatopancreatic and cuticular pigmentation. Prior to challenge, healthy shrimp were acclimatized for seven days in filtered and aerated seawater maintained at 25 ± 1°C and salinity of 8‰. Four different bacterial concentration groups were prepared for the reinjection challenge: 1×10^9, 2.5×10^8, 6.25×10^7, and 1.56×10^7 CFU/mL. A parallel control group received an equivalent volume of sterile physiological saline. Each treatment—including the control—was replicated in triplicate (n = 3 per group). Mortality was recorded every 24 h, and cumulative survival rates were calculated over the 7-day observation period.

DNA extraction, library construction, and sequencing

P. damselae strain MRY0520 is kept in the Institute of Oceanology & Marine Fisheries. The preserved strain was inoculated into liquid LB medium and incubated on a shaker at 25 ℃ for 24 h. Genomic DNA was extracted using HiPure Bacterial DNA Kits (Magen, Guangzhou, China) following the manufacturer's instructions. The integrity and quality of the extracted DNA were assessed by 1% agarose gel electrophoresis and Nanodrop 2000 spectrophotometry, respectively. Subsequently, high-quality genomic DNA libraries were sequenced using the PacBio/Illumina sequencing platform by Genedenovo Biotechnology Co., Ltd. (Guangzhou, China).

Genome assembly and read filtering

Qualified genomic DNA was fragmented using G-tubes (Covaris, Woburn, MA, USA) and end-repaired to prepare SMRTbell DNA template libraries (with fragment sizes greater than 10 kb selected by the BluePippin system, according to the manufacturer's specifications (PacBio, Menlo Park, CA).

Continuous long reads were obtained from SMRT sequencing runs and used for de novo assembly with Falcon (version 0.3.0) (Chin et al. 2016). Raw data from the Illumina platform were filtered using FASTP (version 0.20.0) (Chen et al. 2018) according to the following standards: 1) removal of reads containing ≥10% unidentified nucleotides (N); 2) removal of reads with ≥50% of bases having Phred quality scores ≤20; 3) removal of reads aligned to the barcode adapter. Clean reads were filtered for genome sequence correction to improve assembly quality and determine the final genome sequences using Pilon (version 1.23) (Walker et al. 2014).

Genome component prediction and gene functional annotation

The ORFs (Open reading frames) were predicted using Prokka (Seemann 2014) and subsequently annotated with the NCBI prokaryotic genome annotation pipeline (Tatusova et al. 2016). Noncoding RNAs, including rRNAs, were predicted using rRNAmmer (Lagesen et al. 2007); tRNAs were identified by tRNA-scan (Lowe and Chan, 2016); and sRNAs were identified by cmscan (Nawrocki and Eddy 2013). Genomic islands were predicted using IslandPath-DIMOB (Bertelli and Brinkman 2018). CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) elements were identified with CRISPRFinder (Grissa et al. 2007). Transposons were predicted using the online tool TransposonPSI (http://transposonpsi.sourceforge.net/). Interspersed repeat elements were validated with RepeatMasker (Chen 2004), and tandem repeat elements were confirmed using Tandem Repeats Finder (TRF) (Benson 1999). Prophages were identified using PHAST (Fouts 2006).

Several complementary methods were employed for the annotation of the assembled sequence. Genes were annotated by aligning them with sequences deposited in various protein databases, including the National Center for Biotechnology Information (NCBI) non-redundant protein sequence (Nr) database, UniProt/Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO), and Cluster of Orthologous Groups of proteins (COG). Protein family annotation was performed using Pfam_Scan based on the Pfam database (Finn et al. 2014). Additional annotations were conducted using the following databases: Carbohydrate-Active enZymes (CAZy), Pathogen Host Interactions (PHI), Virulence Factors of Pathogenic Bacteria (VFDB), and the Comprehensive Antibiotic Resistance Database (CARD). antiSMASH was applied to predict gene clusters of secondary metabolites (Blin et al. 2017). Two-component systems (TCSs) were predicted based on their structural characteristics (Cheung and Hendrickson 2010). Secretory proteins were predicted using SignalP 4.0 (Petersen et al. 2011), while transmembrane proteins were predicted using TMHMM (Krogh et al. 2001). Type III secretion systems (T3SS) were predicted using EffectiveT3 (Arnold et al. 2009).

Comparative genomic analysis

Comparative genomic analysis was performed using seven additional whole-genome sequences of common aquatic pathogen strains obtained from the NCBI database (Supplementary Table S1). The filtered gene sets were clustered for each targeted Vibrio species using MUSCLE alignment to compare common single-copy ortholog sequences within each species (Edgar 2004). The results were then analyzed using a super-alignment matrix. Subsequently, a phylogenetic tree was reconstructed using the maximum likelihood method (MLTREE) (Guindon and Gascuel, 2003). Gene family expansion and

contraction were analyzed with CAFE (Computational Analysis of Gene Family Evolution) software following cluster analysis (De Bie et al. 2006). Additionally, gene family functions were further annotated through GO and KEGG analyses. Synteny analysis of protein sequences was conducted with MCScanX between the target and reference genomes (Wang et al. 2012).

Results

The source and histopathological analysis of diseased *L. vannamei*

Healthy *L. vannamei* exhibited high vitality, normal feeding behavior, a black and plump hepatopancreas with well-defined boundaries, and a clear digestive tract. In contrast, diseased individuals showed no significant changes in body color but presented symptoms such as empty stomachs and intestines, along with a shrunken hepatopancreas (Fig. 1). The disease progression from onset to death in affected shrimp is relatively rapid, characterized by sporadic mortalities, which local farmers refer to as “Dixing disease”.

The hepatopancreas of healthy *L. vannamei* is a complex tubular gland composed of numerous hepatopancreatic ducts. The hepatic tubules are tightly arranged, featuring a star-shaped lumen and an intact structure. Bubble cells, fibroblasts, and absorptive cells are clearly distinguishable, with no blood cell infiltration observed between the tubules (Fig. 1B upper). In contrast, significant histopathological changes were observed in the hepatopancreas of diseased *L. vannamei*, mainly manifested as significant enlargement of the tubular lumens and partial shedding of epithelial cells into the lumens. The star-shaped tubular structure disappears, the structure is damaged, some functional cells become indistinguishable, and blood cell infiltration is evident (Fig. 1B lower).



Fig. 1 Comparison of the appearance and histopathological analysis of healthy and diseased *L. vannamei*. (A) Appearance comparison of healthy (left) and diseased (right) *L. vannamei*. (B) Histopathological analysis of the hepatopancreas in healthy (upper) and diseased (lower) *L. vannamei*. Red arrows in the histopathological images indicate B (blister cell), F (fibrillar cell), R (resorptive cell), H (hemocytic infiltration), and T (cell abscission).

Isolation and identification of dominant culturable bacteria from diseased *L. vannamei*

After separation and purification, no bacteria were isolated from the muscle tissue of the diseased shrimp. Four dominant bacterial strains with consistent colony morphology were isolated from the hepatopancreas of the diseased shrimp; these strains differed significantly from those isolated from the hepatopancreas of healthy shrimp. The dominant bacterial strain, designated MRY0520, was identified as *Photobacterium damselae* subsp. *damselae* through morphological analysis, 16S ribosomal DNA (rDNA) sequencing, and physiological and biochemical tests (Peng et al. 2024). The pathogenicity of strain MRY0520 to *L. vannamei* needs to be confirmed through further reinfection experiments.

Results of injection challenge testing with strain MRY0520

The injection reinfection experiment demonstrated that the strain MRY0520 could cause healthy shrimp to die from illness, exhibiting reduced vitality and almost no feeding. The hepatopancreas appeared pale and atrophied with blurred edges and the presence of the jejunum. These symptoms were consistent with those seen in naturally diseased shrimp (Fig. 2A). Subsequently, the dominant bacteria were isolated from the reinfected shrimp hepatopancreas. The resulting dominant strains closely matched MRY0520 in morphological characteristics, physicochemical properties, and 16S rRNA sequencing results, confirming the high pathogenicity of strain MRY0520 (Sussman et al. 2008).

The injection reinfection experiment results showed that *L. vannamei* in the high-concentration bacterial solution injection group exhibited delayed movement, reduced vitality, and a greater tendency to lie at the bottom of the bucket after infection. The 1×10^9 CFU/mL group experienced mortality first, with a 95% death rate at 12 h and complete mortality by 48 h. Only a portion of the 1.56×10^7 CFU/mL group died, while the control group remained in good condition (Fig. 2B). Using the improved Spearman-Karber method (Hagopian-Schlekat et al. 2001), the 72 h median lethal dose for (72 h-LD_{50}) of the bacterium injected and reinfected in *L. vannamei* was calculated to be 2.15×10^5 CFU/g. These results indicate that strain MRY0520 is the pathogenic bacterium responsible for the disease and exhibits strong pathogenicity to *L. vannamei*.

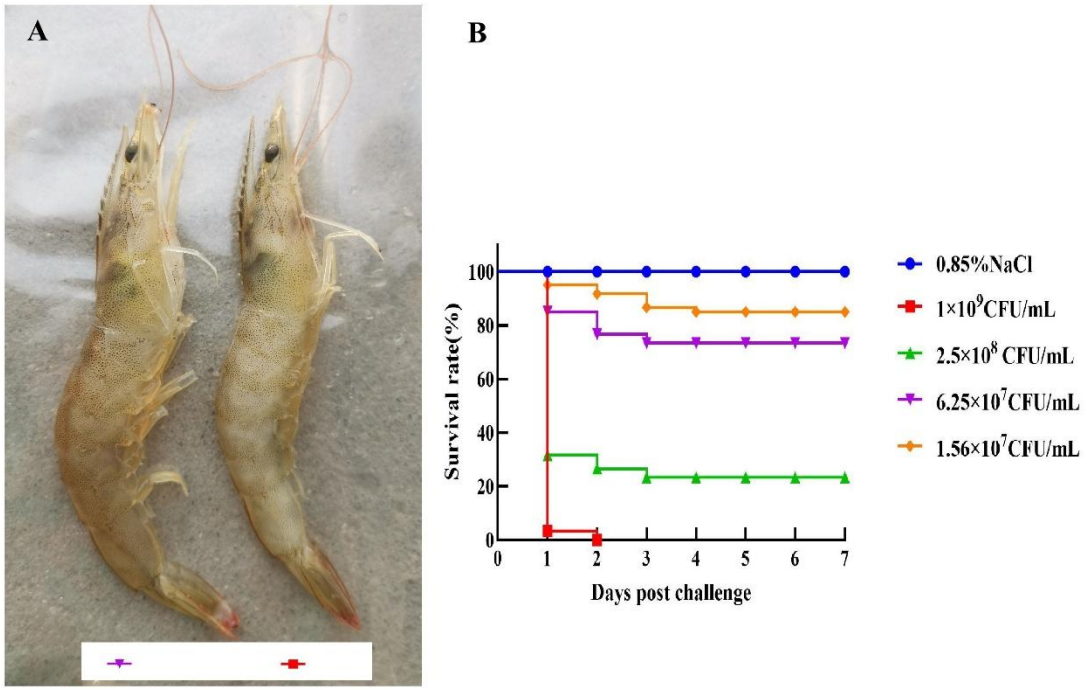
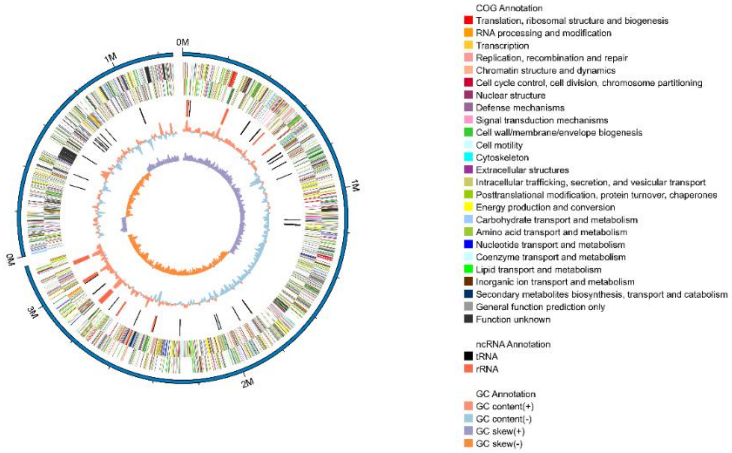


Fig. 2 Results of injection challenge test with strain MRY0520

Sequencing and prediction results

The genome of *P. damselae* strain MRY0520 consists of two chromosomes, totaling 4,451,849 base pairs with a GC content of 40.87% (Supplementary Table S2). It encodes 3,663 genes, spanning 3,691,779 bp, which accounts for 83% of the genome (Supplementary Table S3). The genome includes 208 tRNA genes, 62 rRNA genes (comprising 22 5S rRNA, 20 16S rRNA, and 20 23S rRNA), and two CRISPR loci (CRISPR1: positions 151,541–153,549; CRISPR2: positions 161,979–162,726). Additionally, there are two genomic islands with a combined length of 39,218 bp and an average length of 19,609 bp. No prophages were predicted. The circular genome map of strain MRY0520 is shown in Fig. 3A. Strain MRY0520 genome sequencing also revealed the presence of a plasmid (83782 bp) in the cell (Fig. 3B).

A.



B.

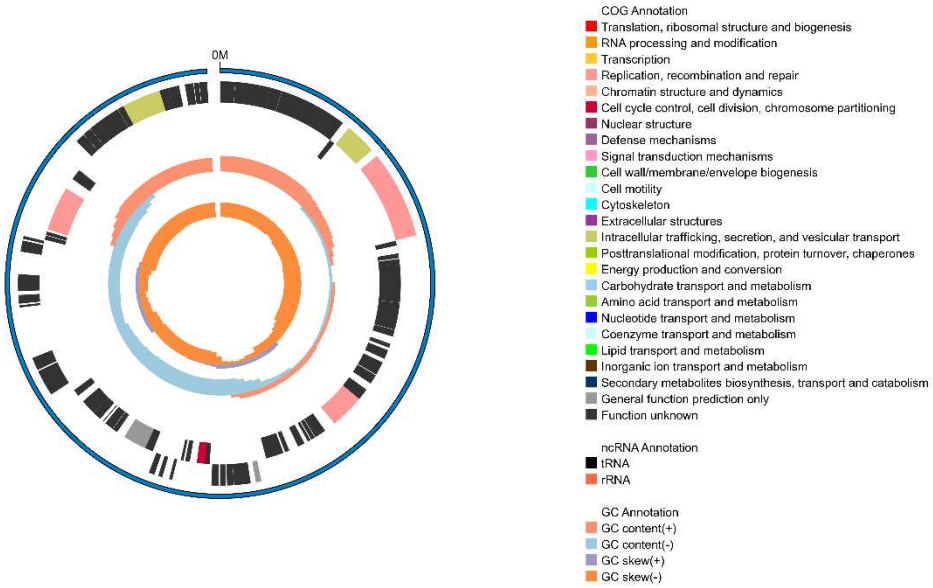


Fig. 3 Circular map of the genome (A) and plasmid (B) of strain MRY0520. The genome position coordinates of the target strain are displayed from the outermost circle inward. The outermost circle represents the position coordinates of the genome sequence. Moving inward, the circles represent: genes on the positive strand, genes on the negative strand, ncRNA (black indicates tRNA, red indicates rRNA), GC content (red indicates values above the mean, blue indicates values below the mean), and GC skew (used to measure the relative content of G and C and to mark the starting and ending points in circular chromosomes). GC skew is calculated as (G - C) / (G + C); purple indicates values greater than 0, and orange indicates values less than 0.

Functional annotation

KEGG is a database used for the systematic analysis of metabolic pathways involving gene products and compounds within cells, as well as the functions of these gene products. A total of 2,399 genes from strain MRY0520 were annotated in the KEGG database and clustered into 23 metabolic pathways based on their functions. Among these, 1,802 genes are related to metabolism, with a significant number involved in carbohydrate, amino acid, cofactor, and vitamin metabolism. Additionally, 203 genes are associated with genetic information processing, including translation, folding, sorting, and degradation. There are 278 genes related to environmental information processing, such as transmembrane transport and signal transduction. Furthermore, 220 genes are linked to cellular processes, including cell movement, growth, and death. Genes associated with biological systems are the fewest, totaling only 11, primarily involved in environmental adaptation and the immune system (Fig. 4). Notably, metabolism-related genes account for 75.11% of the annotated genes in the database. The predominance of genes involved in metabolic pathways supports the strain's metabolic processes for amino acids, energy, nucleotides, and lipids, which are essential for maintaining its vital activities.

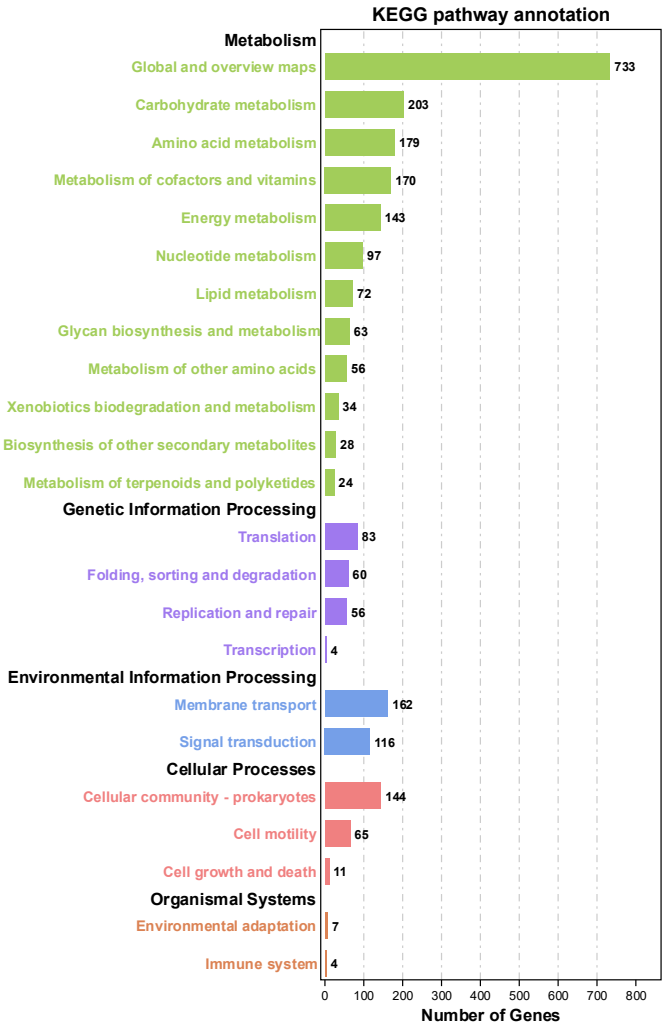


Fig. 4 KEGG enrichment analysis of strain MRY0520

Gene Ontology (GO) is an internationally standardized system for classifying gene functions, providing a comprehensive description of the properties of genes and their products in living organisms. It offers a dynamically updated set of standardized terminology. GO enrichment analysis revealed that the genome of strain MRY0520 contains 5,714 genes associated with molecular function, 3,120 genes related to cellular components, and 9,838 genes involved in biological processes (Fig. 5). Regarding molecular function, many genes are linked to catalytic activity and binding. For cellular components, most genes are associated with cellular anatomical entities. In terms of biological processes, a significant proportion of genes are connected with cellular and metabolic processes. These results indicate that the strain possesses numerous genes related to biological processes, participating in cell division and bacterial metabolism.

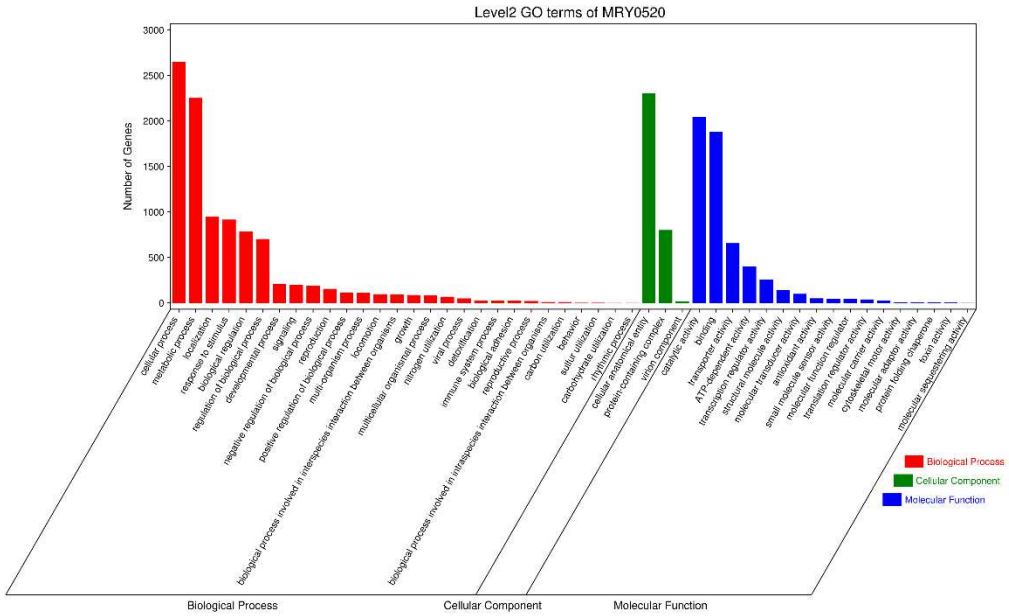


Fig. 5 GO Enrichment Analysis of Strain MRY0520

COG, Cluster of Orthologous Groups of proteins, is organized based on phylogenetic relationships and the encoded proteins found in the complete genomes of bacteria, algae, and eukaryotes. Protein sequences are annotated in the COG database through sequence alignment, and each COG cluster consists of orthologous sequences, allowing for functional inference of the sequences. The strain MRY0520 was annotated with 2,780 genes in the COG database, which were categorized into 21 functional groups. Among these, 188 genes are associated with proteins involved in translation, ribosomal structure, and biotransformation; 1 gene is linked to RNA processing and modification; 231 genes are related to transcription; 155 genes are involved in replication, recombination, and repair; 1 gene is connected to chromatin structure and dynamics; 34 genes are associated with cell cycle control, cell division, and chromosome partitioning; 59 genes are linked to defense mechanisms; 189 genes are involved in signal transduction mechanisms; 195 genes are related to carbohydrate transport and metabolism; 303 genes are associated with amino acid transport and metabolism; 89 genes are involved in nucleotide transport and metabolism; 159 genes are linked to coenzyme transport and metabolism; and 88 genes are associated with lipid transport and metabolism (Fig. 6). These results indicate that the genes of this strain participate to varying degrees in synthesis and metabolic processes, with the greatest involvement observed in amino acid transport and metabolism as well as protein transcription.

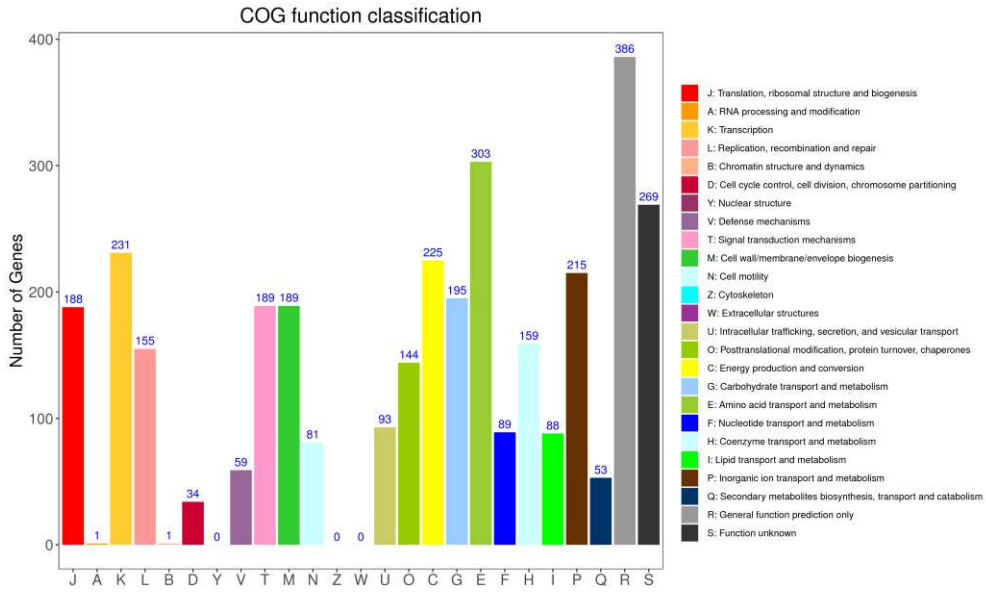


Fig. 6 COG Function Classification Chart of Strain MRY0520

Carbohydrate-Active enZYmes (CAZy) Database is a specialized resource focused on carbohydrate-related enzymes, including families that catalyze carbohydrate degradation, modification, and biosynthesis. The CAZy annotation results for strain MRY0520 are shown in Fig. 7. In total, 496 genes were annotated across six types of active enzymes: 195 glycosyl transferases, 178 glycoside hydrolases, 90 carbohydrate-binding modules, 24 carbohydrate esterases, 5 polysaccharide lyases, and 4 carbohydrate auxiliary activators.

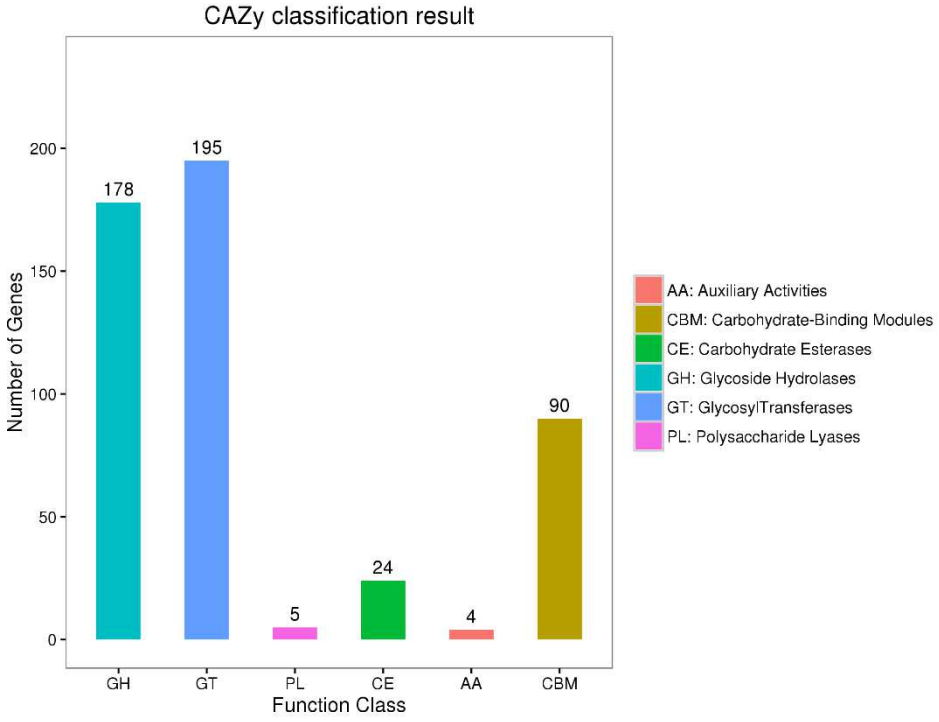


Fig. 7 The CAZy function classification chart of strain MRY0520

Software tools SignalP 4.0 and TMHMM 2.0 were used to predict secreted proteins. Annotations were based on protein sequence-function database (KEGG, Nr), the TNSS (type N secret systems, type

I-V II) related proteins were extracted. Additionally, T3SS effector proteins of Gram-negative bacteria were predicted using EffectiveT3 software. The results identified a total of 3,663 proteins, including 46 T3SS effector proteins, 390 signal peptide proteins, 57 transmembrane proteins, 333 secretory proteins, and 3,330 non-secretory proteins (Supplementary Table S4).

Using the PHI database to analyze the pathogenic genes of strain MRY0520, 97 genes were identified as related to disease. Additionally, mutations in 481 genes (such as the phosphoribosyl transferase gene) reduce the strain's virulence; mutations in 202 genes (including those related to reactive oxygen species) do not affect virulence; mutations in 9 genes (such as the receptor potential ion channel protein gene) result in strain lethality; mutations in 60 genes (such as the amylase gene) lead to loss of pathogenicity; 1 gene (ubiquinone oxidase gene) is associated with chemical targeting; mutations in 69 genes (such as the regulatory protein RsmA gene) enhance virulence; and mutations in 7 genes (including the ipx10 gene) affect bacterial effector protein secretion (Fig. 8).

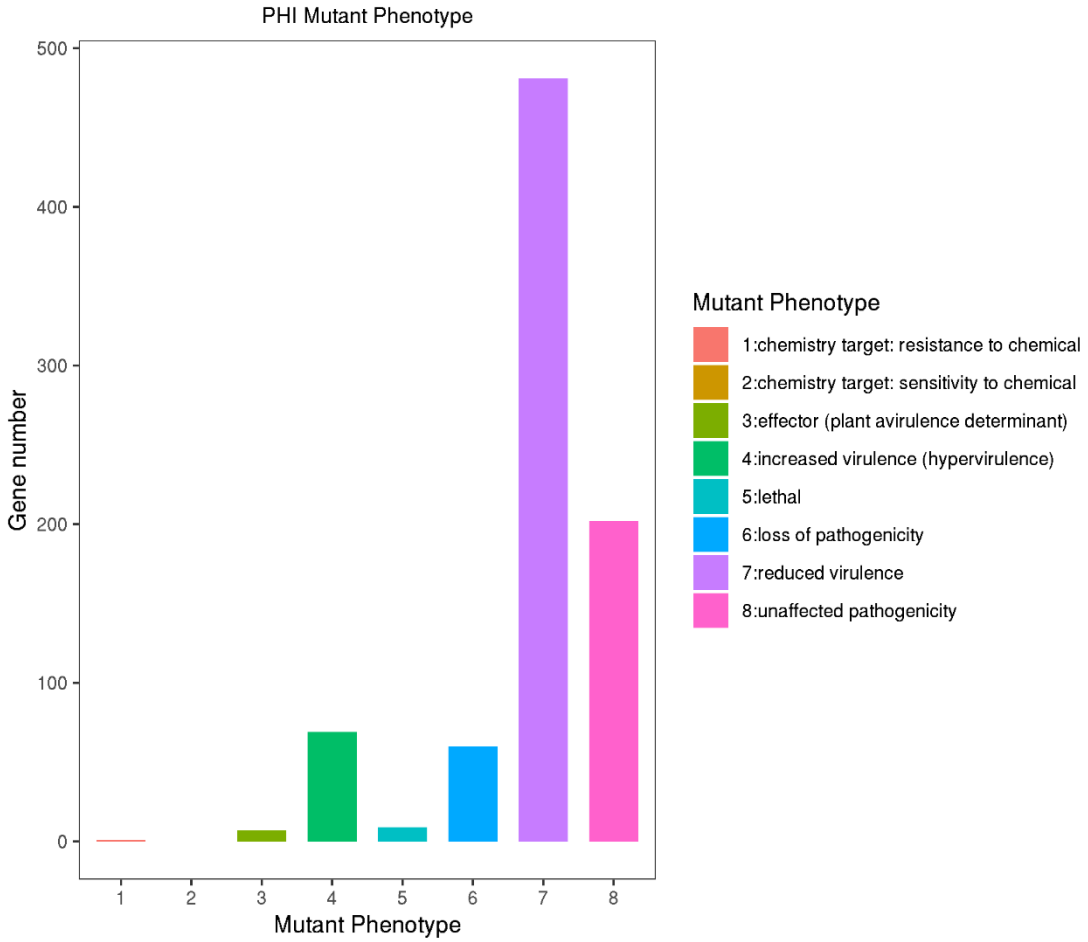


Fig. 8 Classification of pathogenic gene phenotype mutation types of strain MRY0520

VFDB, Virulence Factors of Pathogenic Bacteria, can provide key evidence for the invasion mechanisms of pathogenic bacteria. When integrated with comparative genomics and other analytical approaches, it facilitates the study of virulence factor composition and genome distribution across different pathogenic bacteria within the same genus at the whole-genome level. By comparing the MRY0520 genome with the VFDB database, 133 virulence factor-related genes were identified.

299 Subsequent data filtering resulted in 46 highly reliable virulence genes, including sixteen flagellar-related
300 genes, six chemotaxis-related genes, three RNA polymerase factors, six secretion-related genes, eight
301 metabolism-related genes, and eight other associated genes (Table 1).

302 **Table 1** Annotated virulence factor genes and coding proteins of strain MRY0520.

Order number	Virulence factor gene	Encoding proteins
1	*FlgD*	flagellar basal body rod modification protein D
2	*FlhB*	flagellar biosynthesis protein B
3	*FlgC*	flagellar basal body rod protein C
4	*FliP*	flagellar biosynthesis protein P
5	*FliR*	flagellar biosynthesis protein R
6	*FlgI*	flagellar P-ring protein precursor
7	*FlaA*	flagellin
8	*FlhA*	flagellar biosynthesis protein A
9	*FliE*	flagellar hook-basal body protein
10	*FliN*	flagellar motor switch protein
11	*FlgG*	flagellar basal body rod protein G
12	*FliG*	flagellar motor protein
13	*FilM*	flagellar motor switch protein
14	*FliI*	flagellum-specific ATP synthase
15	*MotA*	flagellar motor protein
16	*FliF*	flagellar M-ring protein
17	*CheR*	chemotaxis methyltransferase
18	*CheY*	chemotaxis protein Y
19	*CheV*	chemotaxis protein V
20	*CheW*	chemotaxis protein W
21	*CheA*	chemotaxis protein A
22	*CheB*	chemotaxis-specific methylesterase
23	*RpoN*	RNA polymerase factor sigma-54
24	*RpoS*	factor of RNA polymerase
25	*HtpB-Hsp60*	HtpB - Hsp60, 60K heat shock protein
26	*GalU*	argininosuccinate lyase
27	*WbfV/WcvB*	WbfV/WcvB - predicted UDP-glucose 6-dehydrogenase
28	*GspD*	general secretion pathway protein D
29	*EpsE*	general secretion pathway protein E
30	*PilB*	type IV pilus assembly protein
31	*Eno*	enolase
32	*IcmF/VasK*	type VI secretion system inner membrane protein

33	*EpsF*	general secretion pathway protein F
34	*FlrA*	sigma-54-dependent transcriptional activator
35	*SigA/RpoV*	RNA polymerase sigma factor
36	*LuxS*	S-ribosylhomocysteinase
37	*ABTJ_03744*	ABTJ_03744 - UDP-glucose-4-epimerase
38	*MsbA*	fused lipid transporter subunits of ABC superfamily
39	*EpsG*	general secretion pathway protein G
40	*MshE*	type 4 fimbrial assembly protein
41	*MrsA/GlmM*	mrsA/glmM - predicted phosphomannomutase
42	*VipB/MglB*	type VI secretion system tubule-forming protein
43	*LpxB*	lipid-A-disaccharide synthase
44	*KdsA*	2-dehydro-3-deoxyphosphooctonate aldolase
45	*MshA*	mannose-sensitive haemaglutinin
46	*GmhA/LpcA*	phosphoheptose isomerase

CARD database was used to analyze the resistance genes of strain MRY0520. The results indicated that its genome contains ten resistance genes, corresponding primarily to the following antibiotics: fluoroquinolones, aminoglycosides, diaminopyrimidines, macrolides, cephalosporins, glycylcyclines, tetracyclines, rifamycins, phenicols, and fosfomycin. Attention should be given to resistance against these antibiotics in clinical applications.

Secondary metabolites are compounds with complex molecular structures and often unclear functions, synthesized from primary metabolites. Generally, genes encoding biosynthetic enzymes in secondary metabolic pathways are organized in clusters on chromosomes. The antiSMASH tool was used to predict secondary metabolite gene clusters (Medema et al. 2011). The results showed that the genome of strain MRY0520 contains a thiopeptide antibiotic gene cluster comprising 18 genes (Fig. 9).



Fig. 9 The secondary metabolic gene clusters chart of strain MRY0520

Phylogenetic reconstruction

To investigate the evolutionary relationships among different species, a phylogenetic tree was constructed using the software IQ-TREE based on the MLTREE (Nguyen et al. 2013). Homologous gene families were analyzed through clustering, and two software programs, MUSCLE and Gblocks, were employed to select single-copy homologous genes for multiple sequence alignment and quality control (MUSCLE and Gblocks) (Edgar 2004; Castresana 2000). Comparative analysis of the genome sequence of strain MRY0520 against GenBank entries in NCBI revealed high similarity with strain Phdp 91-197 (NCBI No. GCA_002356235.1) and strain Phdd Wu-1 (NCBI No. GCA_003130755.1). Strain MRY0520 clustered closely with strain Phdd Wu-1, indicating the closest phylogenetic relationship (Fig. 10).

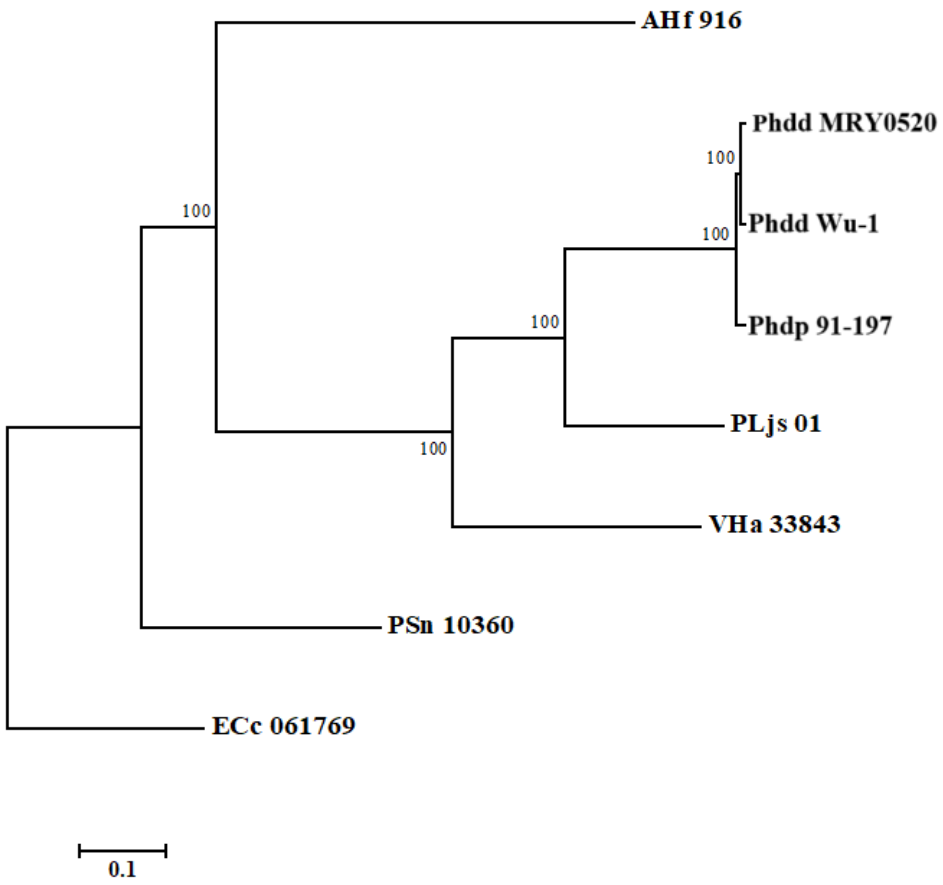
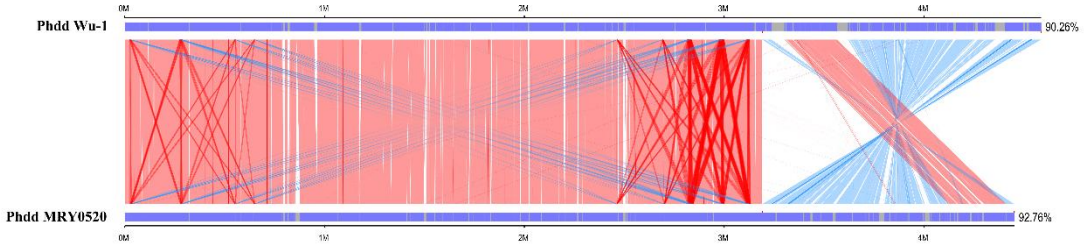


Fig. 10 Phylogenetic tree of eight common aquatic pathogens strains

Comparative genomic analyses

In the previous analysis, strains Phdd MRY0520, Phdd Wu-1, and Phdp 91-197 exhibited the highest genome homology. The whole genome sequences of these three strains were compared pairwise. The target genome and the reference genome were analyzed using the software MUMmer to identify large-scale co-linear relationships between the genomes (Kurtz et al. 2004). Subsequently, the software SyRI was used to compare corresponding functional regions, determine local positional arrangements, and identify the locations of translocations (trans), inversions (inv), and combined translocation-inversion events (Goel et al. 2019). Finally, synteny analysis was performed pairwise among strains MRY0520, Phdd Wu-1, and Phdp 91-197. The results indicated limited synteny between the target strain MRY0520 and the two reference strains (Fig. 11). Although a large number of homologous genes exist between strain MRY0520 and the reference strains, the presence of non-homologous genes suggests that strain MRY0520 possesses unique genomic features.

A.



B.

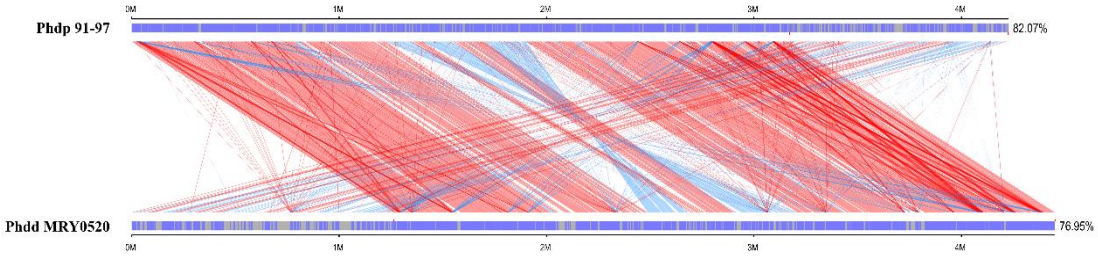


Fig. 11 The results of the synteny analysis are as follows: (A) synteny analysis between strain MRY0520 and strain Phdd Wu-1; (B) synteny analysis between strain MRY0520 and strain Phdp 91-197.

Discussion

Previous reports indicate that *P. damselae* infection in aquatic organisms primarily affects fish, causing surface ulcers and hemorrhagic necrosis (Pedersen et al. 2009; Labella et al. 2010; Zhang et al. 2019). Typical symptoms following infection in *P. monodon* and *E. carinicauda* include hepatopancreas damage accompanied by an empty digestive tract. Phdd, a pathogenic bacterium isolated from the hepatopancreas of diseased *P. monodon* and *E. carinicauda*, was associated with pale hepatopancreas and empty stomachs and guts in the affected shrimp. Additionally, Phdd was isolated from diseased *L. vannamei*, which exhibited the characteristic symptom of "black gills" (Wang et al. 2020). In this study, Phdd strain MRY0520 was isolated from the hepatopancreas of *L. vannamei* affected by “Dixing disease”. The primary symptom in the diseased shrimp was liver and pancreas damage, with no observed "black gill" symptoms, marking a significant difference from previous reports. The LD_{50} values of Phdd for *P. monodon* and *E. carinicauda* were 2.5×10^5 CFU/g and 2.30×10^5 CFU/g respectively (Liu et al. 2016; Vaseeharan et al. 2007), and the LD_{50} value for L. vannamei was previously reported as 9.75×10^5 CFU/g (Wang et al. 2020). However, in this study, the LD_{50} of Phdd strain MRY0520 for *L. vannamei* was 2.15×10^5 CFU/g. Clearly, the MRY0520 strain isolated here exhibits significantly higher acute toxicity to *L. vannamei*.

The pathogenicity and damage caused by Vibrio to aquatic animals are primarily associated with their virulence factors (Cameron et al. 2008). Studies have shown that hemolysin is one of the most important virulence factors of Phdd (Rivas et al. 2011). The isolate MRY0520 discussed in this article carries multiple virulence genes, except for *Dly* (Yu et al. 2024). The virulence gene *Dly* encodes a

hemolysin located on the virulence plasmid pPHDD1, which alters cell membrane permeability and causes the rupture and lysis of red blood cells, resulting in alpha hemolysis; however, its prevalence is very low. Among the four detected genes, *plpV*, *hly*, and *hlyA* are associated with hemolytic activity, while *coIP* is a virulence-enhancing gene (Rivas et al. 2013), further indicating that the Phdd strain MRY0520 exhibits strong pathogenicity.

A certain proportion of low-quality data exists in the raw sequencing data. To ensure the authenticity and accuracy of subsequent information analysis, it is essential to filter and process the raw data from PacBio and Illumina sequencing to obtain clean data. Continuous long reads were obtained from SMRT sequencing runs and assembled de novo using Falcon (version 0.3.0) (Chin et al. 2016). After filtering, the clean reads were used to calibrate the genome sequences to improve assembly quality, with Pilon (version 1.23) (Walker et al. 2014) employed to generate the final genome sequence. Based on the assembled genome and predicted encoding genes, a circular genome diagram was created to comprehensively display genomic features (Fig. 3). This includes the distribution of genes on the positive and negative strands, COG functional annotations, and GC content. Such a comprehensive visualization provides a more thorough and intuitive understanding of the strain's genomic characteristics.

During the infection process of pathogenic bacteria in the host, strains with varying virulence cause different levels of damage to the body. The key factors influencing the pathogenicity of these strains are closely associated with the virulence factors they possess. In this study, numerous genes related to survival and pathogenicity were identified in strain MRY0520 through whole-genome sequencing. Subsequently, the most representative virulence genes were selected for further discussion and analysis.

Flagella are organelles responsible for bacterial motility, comprising the basal body, hook sheath, and filament (Moens and Vanderleyden 1996). Numerous studies have demonstrated a link between flagella and pathogenicity (Duan et al. 2013). For example, mutation of the filament gene in *Edwardsiella tarda* significantly reduced its pathogenicity compared to the wild type, indicating that the filament contributes to the pathogenicity of *E. tarda* (He et al. 2012). Bacterial motility also plays a critical role in the pathogenic infection processes of *Vibrio cholerae* and *Pseudomonas aeruginosa* (Krukonis and DiRita 2003; Arora et al. 2005). In this study, flagellar-related genes were found to be involved in the biosynthesis and motility of flagella. As common virulence factors, these flagellar genes are hypothesized to play a crucial role in the infection of the hepatopancreas by strain MRY0520. To date, few studies have investigated the motility and pathogenic mechanisms of *P. damselae* flagella, and further research is needed to elucidate the specific relationship between flagella and host disease.

To adapt to the complex and variable external natural environment, motile bacteria move toward or away from environmental stimuli to seek benefits and avoid hazards. This behavior is known as bacterial chemotaxis, which helps bacteria find favorable living conditions (Falke et al. 1997). Currently, *Escherichia coli* and *Bacillus subtilis* are the primary models for studying bacterial chemotaxis. Among these, research on *E. coli* chemotaxis is relatively comprehensive. The signal transduction pathway of its chemotactic system mainly comprises three components: membrane receptors known as methyl-accepting chemotaxis proteins (MCPs) that sense the external environment; the chemotaxis signaling system that transmits signals to the flagellar motor via proteins such as CheW, CheA, CheB, and CheY;

and the adaptation mechanism that adjusts the initial input signal (Arora et al. 2005; Keegstra et al. 2022). In our study, six chemotaxis-related genes—*CheA*, *CheB*, *CheR*, *CheV*, *CheW*, and *CheY*—were identified in strain MRY0520. However, the specific signal transduction pathway in this strain remains undetermined and requires further analysis and investigation.

Through whole-genome sequencing, we identified two primary RNA polymerase factors present in strain MRY0520: rpoN and rpoS. RpoN, also known as sigma-54, was first characterized in *E. coli* as a subunit of RNA polymerase that regulates the expression of nitrogen metabolism-related genes. Additionally, RpoN has been shown to regulate carbon metabolism-related genes in *Listeria monocytogenes* (Arous et al. 2004). RpoS, also known as sigma-38, mainly plays a critical role under stress conditions. Some studies have reported that rpoS negatively regulates the expression of genes involved in flagellar synthesis and motility. For example, under stress conditions, the *rpoS* gene in *E. coli* is upregulated, leading to inhibition of flagellar synthesis and loss of bacterial motility (Shi et al. 1993). Conversely, under normal conditions, rpoS is not expressed, and bacterial motility is enhanced (Dong et al. 2011). Both *rpoN* and *rpoS* participate in the synthesis and chemotactic regulation of bacterial flagella [51]. The transcriptional regulation of related genes is essential for bacterial survival and involves multiple regulators working together, often through complex regulatory networks, to respond to external stimuli. Therefore, it remains to be determined whether *rpoN* and *rpoS* also play regulatory roles in *P. damselae* and what their regulatory networks might be. These questions require further experimental investigation.

Conclusions

The impact of *P. damselae*, as a novel pathogen affecting shrimp, on the shrimp industry requires further evaluation but warrants significant attention. Therefore, its genomic information, functional annotation of key genes, and insights into its evolutionary and developmental relationships have become essential for understanding the mechanisms of pathogen infection, prevention, and control. Whole-genome sequencing and annotation of strain MRY0520 provide a comprehensive understanding of the pathogen's genetic content, composition, and functions, as well as elucidate its predicted biological and molecular processes. Although strain MRY0520 exhibits conditional pathogenicity similar to some pathogenic bacteria within the Vibrionaceae family (Supplementary Table S1), our results indicate that the genetic relationship among all *P. damselae* strains within the genus *Photobacterium* is relatively close. Comparative genomic analysis of strain MRY0520 with other *P. damselae* strains offers evidence to clarify their phylogenetic relationships. Our findings show that strain MRY0520 shares the highest homology with strain Phdd Wu-1, followed by strain MRY0520 and strain Phdp 91-197. These strains exhibit a high degree of genetic synteny, suggesting that genetic recombination or chromosomal variation occurred during the evolutionary divergence of *P. damselae* strains from other Vibrionaceae strains originating from a common ancestor. The genomic sequence map of strain MRY0520 significantly supplements the rapidly expanding genomic data of the *P. damselae* family, thereby enhancing our understanding of prokaryotic genome diversity and evolution.

Supplementary Materials:

Table S1｜Information of complete genomes of eight common aquatic pathogens strains

Name	Abbreviation	NCBI ID	Predicted gene number
Photobacterium damselae subsp. *damselae* MRY0520	Phdd MRY0520	CP113238-CP113240	3663
Photobacterium damselae subsp. *damselae* Wu-1	Phdd Wu-1	GCA_003130755.1	4446
Photobacterium damselae subsp. *piscicida 91-197*	Phdp 91-197	GCA_002356235.1	4963
Aeromonas hydrophila FDAARGOS_916	AHf 916	GCA_016026875.1	4387
Plesiomonas shigelloides NCTC10360	PSn 10360	GCA_900087055.1	3057
Vibrio harveyi ATCC 33843	VHa 33843	GCA_000770115.2	5342
Photobacterium leiognathi JS01	PLjs 01	GCA_002631085.2	4528
Escherichia coli CFSAN061769	ECc 061769	GCA_003399775.2	5132

Table S2｜Genome assembly results of strain MRY0520

Strain	Genome size(bp)	Correction(bp)	Insertion	Deletion	Substitution	GC(%)
MRY0520	4451849	1835	1118	401	316	40.87

Table S3｜Results of encoding genes prediction in strain MRY0520

Strain	Total Number	Total Length	Maximun Length	Minimum Length	GC(%)
MRY0520	3663	3691179	21681	93	41.70

Table S4｜Prediction and statistics of secreted proteins in strain MRY0520

Strain	Total Protein	SignalP	Transmembrane P	Secret P
MRY0520	3663	390	57	333

Funding This work was supported by research grants from the Earmarked Fund for Jiangsu Agricultural Industry Technology System (JATS［2023］380), Jiangsu Seed Industry Revitalization Project (JBGS[2021]122).

Author contribution L. W., H. L., and X. W. designed the study. Z. Y., Q. X., W. S., H. L.， Y. Y. and K. Fu. performed the experiments. Z. Y. and Q. X. analyzed the data, L. W. and Z. Y. wrote the manuscript. L. W. and X. W. revised and edited the manuscript. X. W. acquired the Funding and supervised the study.

Data availability The data that support the results of the present study are available from the corresponding author upon reasonable request.

Competing interests The authors declare no competing interests.

References

Arnold R, Brandmaier S, Kleine F, Tischler P, Heinz E, Behrens S, Niinikoski A, Mewes HW, Horn M, Rattei T (2009) Sequence-based prediction of type III secreted proteins. PLoS Pathog 5(4): e1000376

Arora SK, Neely AN, Blair B, Lory S, Ramphal R (2005) Role of motility and flagellin glycosylation in the pathogenesis of *Pseudomonas aeruginosa* burn wound infections. Infect Immun 73(7): 4395-4398

Arous S, Buchrieser C, Folio P, Glaser P, Namane A, Hebraud M, Hechard Y (2004) Global analysis of gene expression in an *rpoN* mutant of *Listeria monocytogenes*. Microbiology 150, 1581-1590

Baseggio L, Rudenko O, Buller N, Landos M, Englestädter J, Barnes AC (2021) Complete, closed and curated genome sequences of *Photobacterium damselae* subsp. *piscicida* isolates from Australia indicate mobilome-driven localized evolution and novel pathogenicity determinants. Microb Genom *7*(4): 000562

Benson G (1999) Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res 27(2): 573-580

Bertelli C, Brinkman FS (2018) Improved genomic island predictions with Island Path-DIMOB. Bioinformatics 34(13): 2161-2167

Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, De los Santos ELC, Kim HU, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH (2017) antiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res 45(W1): W36-W41.

Cameron DE, Urbach JM, Mekalanos JJ (2008) A defined transposon mutant library and its use in identifying motility genes in *Vibrio cholerae*. PNAS, *105*(25): 8736-8741.

Chen N (2004) Using Repeat Masker to identify repetitive elements in genomic sequences. Curr Protoc Bioinform *5*(1): 4-10

Cardiff RD, Miller CH, Munn RJ (2014) Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harb Protoc 6: pdb-prot073411

Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17(4): 540-552

Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformat 34(17): i884-90.

Cheung J, Hendrickson WA (2010) Sensor domains of two-component regulatory systems. Curr Opin Microbiol 13(2): 116-123

Chin CS, Peluso P, Sedlazeck FJ, Nattestad M, Concepcion GT, Clum A, Dunn C, O'Malley R, Figueroa-Balderas R, Morales-Cruz A, Cramer GR, Delledonne M, Luo C, Ecker JR, Cantu D, Rank DR, Schatz MC (2016) Phased diploid genome assembly with single-molecule real-time sequencing. Nat Methods 13(2): 1050-1054

Chiu TH, Kao LY, Chen ML (2013) Antibiotic resistance and molecular typing of *Photobacterium damselae* subsp. *damselae*, isolated from seafood. J Appl Microbiol 114(4): 1184-1192

De Bie T, Cristianini N, Demuth JP, Hahn MW (2006) CAFE: a computational tool for the study of gene family evolution. Bioinformatics 22(10): 1269-1271

Dong T, Yu R, Schellhorn H (2011) Antagonistic regulation of motility and transcriptome expression by RpoN and RpoS in *Escherichia coli*. Mol Microbiol 79(2): 375-386

Duan Q, Zhou M, Zhu L, Zhu G (2013) Flagella and bacterial pathogenicity. J Basic Microbiol 53(1): 1-8

Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC bioinformatics 5(1): 113

Falke JJ, Bass RB, Butler SL, Chervitz SA, Danielson MA (1997) The two-component signaling pathway of bacterial chemotaxis: a molecular view of signal transduction by receptors, kinases, and adaptation enzymes. Annu Rev Cell Dev Biol 13(1): 457-512

Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, Sonnhammer ELL, Tate J, Punta M (2014) Pfam: the protein families database. Nucleic Acids Res 42(D1): D222-D230

Fouts DE (2006) *Phage_Finder*: automated identification and classification of prophage regions in complete bacterial genome sequences. Nucleic Acids Res 34(20): 5839-5851

Goel M, Sun H, Jiao WB, Schneeberger K (2019) SyRI: finding genomic rearrangements and local sequence differences from whole-genome assemblies. Genome Biol. 20(1): 277

Grissa I, Vergnaud G, Pourcel C (2007) CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 35(suppl_2): W52-W57

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52(5): 696-704

Hagopian-Schlekat T, Chandler GT, Shaw TJ (2001) Acute toxicity of five sediment-associated metals, individually and in a mixture, to the estuarine meiobenthic harpacticoid copepod *Amphiascus tenuiremis*. Mar Environ Res 51(3): 247-264

He Y, Xu T, Fossheim LE, Zhang XH (2012) FliC, a flagellin protein, is essential for the growth and virulence of fish pathogen *Edwardsiella tarda*. PloS ONE 7(9): e45070

Keegstra JM, Carrara F, Stocker R (2022) The ecological roles of bacterial chemotaxis. Nat Rev Microbiol 20(8): 491-504

Krogh A, Larsson B, Von Heijne G, Sonnhammer ELL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305(3): 567-580

Krukonis ES, DiRita VJ (2003) From motility to virulence: sensing and responding to environmental signals in *Vibrio cholerae*. Curr Opin Microbiol 6(2): 186-190

Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL (2004) Versatile and open software for comparing large genomes. Genome Biol 5(2): R12

Labella A, Sanchez-Montes N, Berbel C, Aparicio M, Castro D, Manchado M, Borrego JJ (2010) Toxicity of *Photobacterium damselae* subsp. *damselae* strains isolated from new cultured marine fish. Dis Aquat Organ 92(1) 31-40

Lagesen K, Hallin P, Rødland EA, Stærfeldt HH, Rognes T, Ussery DW (2007) RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35(9): 3100-3108

Liu F, Liu G, Li F (2016) Characterization of two pathogenic *Photobacterium* strains isolated from *Exopalaemon carinicauda* causing mortality of shrimp. Aquaculture 464: 129-135

Love M, Teebken-Fisher D, Hose JE, Farmer III JJ, Hickman FW, Fanning GR (1981) *Vibrio damsela*, a marine bacterium, causes skin ulcers on the damselfish *Chromis punctipinnis*. Science 214(4525): 1139-1140

Medema MH, Blin K, Cimermancic P, De Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R (2011) antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 39(suppl_2): W339-W346

Moens S, Vanderleyden J (1996) Functions of bacterial flagella. Crit Rev Microbiol 22(2): 67-100

Nawrocki EP, Eddy SR (2013) Infernal 1.1: 100-fold faster RNA homology searches. Bioinformatics 29(22): 2933-2935

Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32(1): 268-274

Pedersen K, Skall HF, Lassen-Nielsen AM, Bjerrum L, Olesen NJ (2009) *Photobacterium damselae* subsp. *damselae*, an emerging pathogen in Danish rainbow trout, *Oncorhynchus mykiss* (Walbaum), mariculture. J Fish Dis 32(5): 465-472

Peng X, Tu H, Yao X, Lan X, Zhong Z, Luo J, Tang Q, Yi S, Xia Z, Yang G (2024) Isolation, identification, and virulence gene analysis of pathogenic *Aeromonas dhakensis* in *Macrobrachium rosenbergii* and histopathological observation. J Ocean Limn 42(2): 664-675

Petersen TN, Brunak S, Von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8(10): 785-786

Potvin E, Sanschagrin F, Levesque RC (2008) Sigma factors in *Pseudomonas aeruginosa*. Fems Microbiol Rev 32(1): 38-55

Rivas AJ, Balado M, Lemos ML, Osorio CR (2011) The *Photobacterium damselae* subsp. *damselae* hemolysins damselysin and HlyA are encoded within a new virulence plasmid. Infect Immun 79(11): 4617-4627

Rivas AJ, Lemos ML, Osorio CR (2013) *Photobacterium damselae* subsp. *damselae*, a bacterium pathogenic for marine animals and humans. Front Microbiol 4: 283

Seemann T (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30(14): 2068-2069

Shi W, Li C, Louise CJ, Adler J (1993) Mechanism of adverse conditions causing lack of flagella in *Escherichia coli*. J Bacteriol 175(8): 2236-2240

Sussman M, Willis BL, Victor S, Bourne DG (2008) Coral pathogens identified for white syndrome (WS) epizootics in the Indo-Pacific. PLoS one, 3(6): e2393

Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44(14): 6614-6624

Thyssen A, Grisez L, Van Houdt R, Ollevier F (1998) Phenotypic characterization of the marine pathogen *Photobacterium damselae* subsp. *piscicida*. Int J Syst Evol 48(4): 1145-1151

Vaseeharan B, Sundararaj S, Murugan T, Chen JC (2007) *Photobacterium* damselae ssp. damselae associated with diseased black tiger shrimp *Penaeus monodon* Fabricius in India. Lett Appl Microbiol 45(1): 82-86

Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM (2014) Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PloS ONE 9(11): e112963

Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, Lee TH, Jin H, Marler B, Guo H, Kissinger JC, Paterson AH (2012) *MCScanX*: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res *40*(7): e49-e49

Wang Z, Shi C, Wang H, Wan X, Zhang Q, Song X, Li G, Gong M, Ye S, Xie G, Huang J (2020) A novel research on isolation and characterization of *Photobacterium damselae* subsp. *damselae* from Pacific white shrimp, *Penaeus vannamei*, displaying black gill disease cultured in China. J. Fish Dis. 43(5): 551-559

Wang L, Xu Q, Yu Z, Hu Z, Li H, Shi W, Wan X (2024) Transcriptomic Analysis Provides Insights into the Energetic Metabolism and Immune Responses in *Litopenaeus vannamei* Challenged by *Photobacterium damselae* subsp. *damselae*. Fishes *9*(9): 350

Yu Z, Wang L, Li H, Shi W, Zhao R, Wan X (2024) Study on “Dixing disease” of *Litopenaeus vannamei* cultured in small shed. Acta Agriculturae Shanghai 40(2):94-101

Zhang Z, Yu YX, Wang K, Wang YG, Jiang Y, Liao MJ, Rong XJ (2019) First report of skin ulceration caused by *Photobacterium damselae* subsp. *damselae* in net-cage cultured black rockfish (*Sebastes schlegeli*). Aquaculture 503: 1-7