



Biochemical and Molecular Characterization of Pathogenic *Vibrio* Associated with White Feces Syndrome in *Penaeus vannamei* and their Antibiotic Susceptibility

Geetha S¹, Madhuri M², Sahithi M³, Askara Y⁴, Sunil Kumar D⁵, Janakiram P^{6*}

^{1,2,3,4,5,6} Department of Marine Living Resources, Andhra University, Visakhapatnam, Andhra Pradesh, India.

*Corresponding Author—E mail: pjram9999@gmail.com

Abstract:

White gut disease (WGD) is an emerging threat to shrimp aquaculture, significantly impacting production and profitability. This study aimed to investigate the bacterial diversity associated with WGD in shrimp and antibiotic susceptibility. The total bacterial count (TBC) and total *Vibrio* count (TVC) were determined in digestive tracts and haemolymph of both Normal and WGD-affected shrimp. The TBC and TVC in the digestive tracts of WGD-affected shrimp were substantially higher (1.5×10^8 CFU/g and 1.41×10^7 CFU/g) compared to Normal shrimp (2×10^5 CFU/g and 0.43×10^4 CFU/g). Similarly, in haemolymph, TBC and TVC in WGD-affected shrimp (0.6×10^8 CFU/ml and 0.62×10^6 CFU/ml) were elevated relative to healthy shrimp (0.51×10^5 CFU/ml and 0.27×10^5 CFU/ml). A total of seven distinct bacterial isolates (V1-V7) were isolated from WGD-affected shrimp and characterized based on morphological, biochemical, and molecular analyses. Molecular characterization through 16S rRNA gene sequencing confirmed species as *Providencia stuartii*, *Vibrio campbellii*, *Vibrio alginolyticus*, *Shewanella algae* and *Shewanella carassii*. *Shewanella carassii* was identified as the same species but with different strains. Antibiotic susceptibility testing revealed isolates *Vibrio campbellii*, *Vibrio alginolyticus*, *Shewanella carassii* exhibited resistance to Penicillin-G, and *Vibrio alginolyticus*, *Shewanella carassii* resistance to Ampicillin whereas *Providencia stuartii* exhibited resistance to Tetracycline and Furazolidone. These findings highlight the pathogenic bacterial community associated with WGD and underscore the need for effective disease management strategies in shrimp aquaculture.

Key words: White gut disease (WGD), Shrimp, Aquaculture, *Vibrio*, Antibiotic, 16S rRNA sequencing.

Introduction:

In Shrimp aquaculture is a vital global food sector driven by rising demand (FAO, 2020) and technological advancements (Boyd et al., 2018). India, a major producer, exported a record 17,81,602 MT of seafood in 2023-24, with vannamei shrimp production at 10,76,970 MT, 70% from Andhra Pradesh (MPEDA). Despite profitability, disease outbreaks, environmental issues, and market fluctuations pose major challenges (Lightner, 2011). Shrimp aquaculture faces major losses from bacterial and viral diseases, with white feces syndrome (WFS) causing up to a 60% production loss (Lightner, 1996; Tangprasittipap et al., 2013) and first detected in Thailand (2010–2011), leading to reduced feed intake and mortality (Chaweepack et al., 2015). characterized by floating white fecal matter, it resulting from hepatopancreatic microvilli sloughing into vermiform bodies, typically appearing 50–60 days post-stocking (Sriurairatana et al., 2014). It was initially linked to microsporidian infection, later studies did not support this hypothesis (Tang et al., 2016).

Vibriosis is a major threat to global shrimp aquaculture, affecting numerous penaeid species (Baticados et al., 1990). WFS is linked to *V. anguillarum*, *V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi*, *V. penaeicida*, and *V. campbellii* causing severe mortality under stress (Lightner, 1988). WGD caused significant mortality, with outbreaks associated with *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus*, and *V. splendidus* (Janakiram et al., 2018). WFS-infected shrimp exhibited greater *Vibrio* diversity, with *V. vulnificus*, *V. fluvialis*, *V. parahaemolyticus*, *V. alginolyticus*, *V. mimicus*, *V. cholerae*, and *V. damsela* found at twice the levels observed in healthy shrimp (Somboon et al., 2012). Identification of beneficial gut microflora in *P. vannamei* may aid disease prevention. *Vibrio campbellii*, a major shrimp pathogen, closely resembles *V. harveyi* but is genetically distinct (Austin & Zhang, 2006; Thompson et al., 2007). Gut microbiota play a key role in shrimp health, with *Shewanella* and *Psychrobacter* found in specific conditions (Ghonimy & Chang, 2024). WFS-affected shrimp show dominant *Vibrio*, *Candidatus Bacilloplasma*, and *Acinetobacter*, *Shewanella* was also dominant in WFS-affected shrimp, following *Vibrio*, *Candidatus Bacilloplasma*, and *Acinetobacter* (Wang et al., 2024). Most research has focused on microbial communities in water and diet effects on intestinal microbiota (Zhang et al., 2014; Tang et al., 2014). Widespread antibiotic use in aquaculture has led to rising resistance, posing global concerns, especially with prophylactic use (Holmström et al., 2003; Chomwong et al., 2018). *Vibrio harveyi*'s resistance challenges shrimp farms, while luminous vibriosis remains uncontrollable despite antibiotics (Jayasree et al., 2008; Shafiqur et al., 2009). Overuse of antibiotics fosters resistance and enables gene transfer between aquatic and human environments (Cabello, 2006). Proper culture management helps prevent bacterial diseases (Flegel, 2012). Therefore, in the present paper efforts have been made to isolate and characterize the bacteria associated with white feces syndrome affected shrimp, *P. vannamei*.

Materials And Methods:

Sample Collection

Normal and White Feces Syndrome(WFS) affected shrimps were collected from culture ponds near koppugundupalem, Rambilli, Visakhapatnam, Andhra Pradesh, India and brought to the laboratory under continuous oxygenated condition. Shrimp were anaesthetized on ice for 5-10 min, and aseptically dissected their digestive tracts (Hp & Gut). Pre-weighed digestive tracts (0.5g) were homogenized with 2% sterile NaCl solution.

Microbiological studies :

Total bacterial count (TBC) and Total *Vibrio* count (TVC) (digestive tracts) & (Haemolymph):

Homogenized samples were serially diluted (10^{-1} to 10^{-6}) in sterile normal saline. One ml from 10^{-3} and 10^{-5} dilutions was added to Petri dishes, followed by lukewarm Zobell's Marine Agar (ZMA) for TPC (Fig.1) and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar for TVC (Fig.2). The plates were gently rotated in both clockwise directions, solidified, inverted, and incubated at $32 \pm 1^\circ\text{C}$ for 24 hours. Colony counts were determined using a colony counter and expressed in CFU/g. Haemolymph (0.5 ml) was drawn from Normal and WFS-affected shrimp and enriched in 2 ml Nutrient Broth. Samples were then pour-plated on Zobell's Marine Agar (ZMA) for TPC and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar for TVC and incubated at 32°C for 18–24 hours. Colonies were counted (CFU/ml) and Total bacterial and *Vibrio* loads were estimated following standard methods (APHA 1992).

Predominant and morphologically different colonies were selected and purified on slants. The isolated bacteria were identified based on morphological, biochemical and molecular characteristics Bergey's Manual of Systematic Bacteriology up to species level (Kreig & Holt, 1984).

Molecular characterization of bacterial isolates through 16S rRNA DNA Extraction and 16S rRNA PCR

Amplification:

Genomic DNA was extracted using the phenol-chloroform method (Sambrook et al., 1989). Bacterial cultures were grown in LB broth, lysed with lysozyme and Proteinase K, and DNA was purified. Extracted DNA was confirmed via 1% agarose gel electrophoresis (Fig.3). PCR amplification of 16S rRNA genes was performed using specific primers in a 40 μL reaction mix. The thermal cycling profile included 35 cycles: denaturation (95°C , 30s), annealing (53°C , 30s), extension (72°C , 1 min), and final extension (72°C , 10 min) (Roux, 1995). Amplified products were analysed on agarose gel under UV light.

PCR Purification, Sequencing, and Phylogenetic Analysis:

PCR products were purified using the ExoSAP-IT® Kit and sequenced bidirectionally with the BigDye Terminator v3.1 Kit on an ABI 3730xl Genetic Analyzer. The 16S rRNA gene sequences were identified using BLAST (NCBI), and the top ten matches were aligned with CLUSTAL W 1.6. A phylogenetic tree was constructed (Fig.5 to 11) using MEGA 7 (Kumar et al., 2015) with the Neighbor-Joining method (Saitou & Nei, 1987). Bootstrap analysis (1000 replicates) ensured reliability (Felsenstein, 1985), and evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura et al., 2004).

Antibiotic sensitivity tests:

The sensitivity of bacterial isolates to various antibiotics was tested following the method of (Bauer et al. 1996). Young cultures (18 hrs.) of the isolates were spread evenly over Mueller's Hinton Agar (MHA) dishes. Twelve commercially available Antibiotic discs (Himedia, Mumbai), Oxytetracycline(O), Norfloxacin(NX), Streptomycin(S), Ciprofloxacin(Cf), Gentamycin(Gen), Ampicillin (A), Furazolidone (Fr), Penicillin-G (P), Kanamycin(k), Chloramphenicol (c), Tetracycline (T) and Erythromycin (E) were placed over the spread culture carefully (6 discs per dish) and incubated overnight at 320°C . Upon incubation, the inhibition zones obtained around the discs were measured by using Kirby Bauer scale (Fig.11).

Results And Discussion:

Bacterial count

TBC and TVC in Normal shrimp digestive tracts were 2×10^5 CFU/g and 0.43×10^4 CFU/g, while in WGD-affected shrimp were 1.5×10^8 CFU/g and 1.41×10^7 CFU/g. In haemolymph, TBC and TVC in Normal shrimp were 0.51×10^5 CFU/ml and 0.27×10^5 CFU/ml, whereas WGD-affected shrimp TBC and TVC were 0.6×10^8 CFU/ml and 0.62×10^6 CFU/ml (Fig.3).

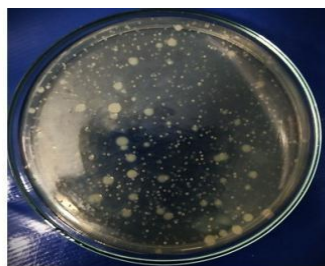


Fig.1 Bacterial colonies on ZMA Agar

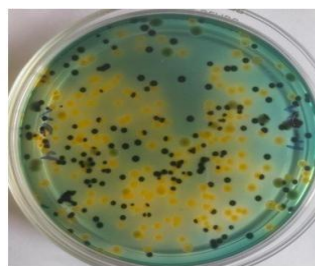


Fig.2 Bacterial colonies on TCBS Agar

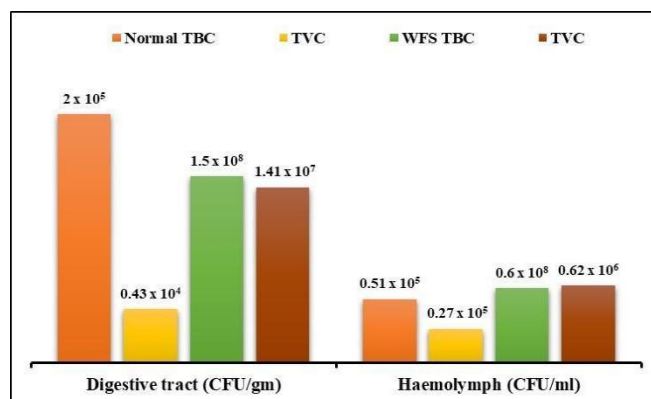


Fig. 3 TBC and TVC of Normal and WFS shrimp

Morphological and biochemical characterization:

Seven distinct bacterial isolates (V1 to V7) were obtained from the digestive tracts of White Gut-affected shrimp which exhibited green coloration, a shiny appearance, opaque optical properties, smooth texture, entire margins, and raised elevations. All seven isolates were examined under a microscope and subjected to the tests like Grams staining, Motility, Aerobic and Anerobic, Oxidative and Fermentative, Acid production from glucose, Oxidase, Catalase, Growth on TCBS, NaCl tolerance test, Temperature tolerance test, Voges Proskauer's test, Arginine, Salt tolerance 1%, ONPG, Citrate, Ornithine, Mannitol, Arabinose, Sucrose, Glucose, Salicin, Cellobiose following methods of Bergey's manual of Systemic Bacteriology (Kreig & Holt, 1984). Based on the above tests isolated bacteria were identified as V1- *Providencia*, V2-*vibrio*, V3-*vibrio*, V4-*shewanella*, V5-*shewanella*, V6-*shewanella*, V7- *shewanella*.

Molecular characterization :

The seven isolates V1 to V7 were subjected to molecular characterization for confirmation of the species through 16S rRNA gene sequencing.

PCR amplification of 16s rRNA gene from isolates :

The amplification of a fragment of the 16s rDNA gene was achieved using the 27F and 1492 primers. A single discrete PCR amplicon band measuring 1500 bp. (Fig.4)

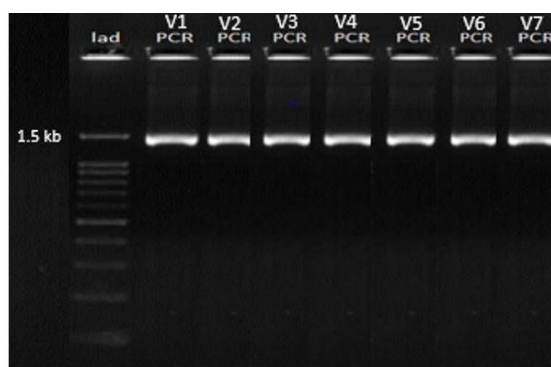
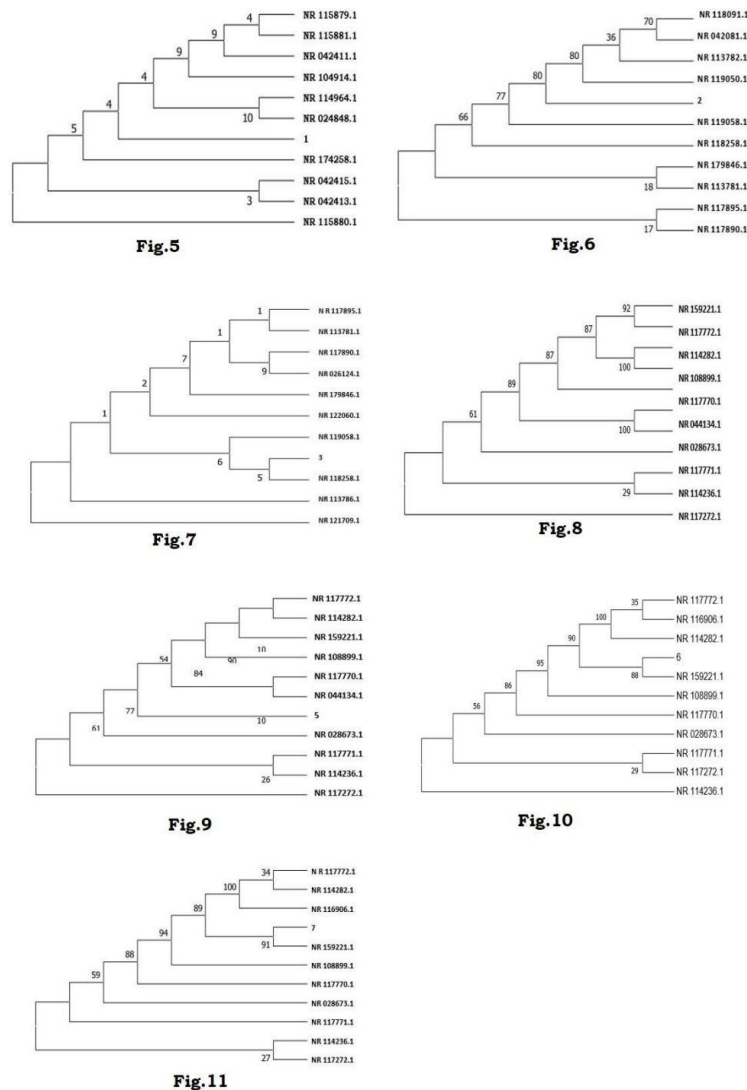


Fig.4 Photograph of gel showing the amplified DNA of 7 isolates

Consensus sequence of 16S rRNA gene was generated from forward and reverse sequence. The analysis involved 11 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of Minimum 1173 to Maximum 1521 positions in the final dataset of

seven isolates. Phylogenetic Evolutionary tree was constructed using MEGA 7. BLAST analysis of the 16S rRNA gene sequences identified the bacterial isolates with high sequence identity: *Providencia stuartii* (96.03%), *Vibrio campbellii* (94.63%), *Vibrio alginolyticus* (96.89%), *Shewanella carassii* (99.43%, 99.08%, 99.86%), and *Shewanella algae* (96.96%). (Fig5-11)



Figs. 5-11. The phylograms of the Isolate V1, V2, V3, V4, V5, V6 and V7 and their similarity with *Providencia stuartii*, *Vibrio campbellii*, *Vibrio alginolyticus*, *Shewanella carassii*, *Shewanella algae*, *Shewanella carassii*, and *Shewanella carassii*.

Based on biochemical analysis, sequence homology, and phylogenetic studies, the bacterial isolates V1, V2, V3, V4, V5, V6, and V7 were confirmed as *Providencia stuartii*, *Vibrio campbellii*, *Vibrio alginolyticus*, *Shewanella carassii*, *Shewanella algae*, *Shewanella carassii*, and *Shewanella carassii*, respectively. The presence of *Shewanella carassii* in three isolates suggests the identification of different strains of the same species and submitted to NCBI gen bank and obtained accession numbers viz. PV174508, PV174516, PV174524, PV174536, PV174537, PV174538 and PV174540.

Antibiotic sensitivity

The inhibition zones observed in the sensitivity assay are illustrated were classified as highly sensitive (>20mm), moderately sensitive (15-20mm), sensitive (10-15mm), and R-resistant (0mm). (Figs.12-13).

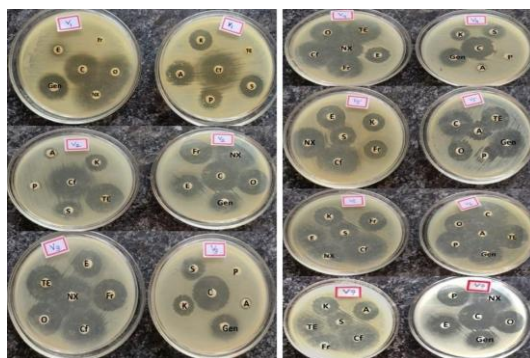


Fig.12 Inhibition zones of different commercial antibiotics against the microbial isolates V1-V7

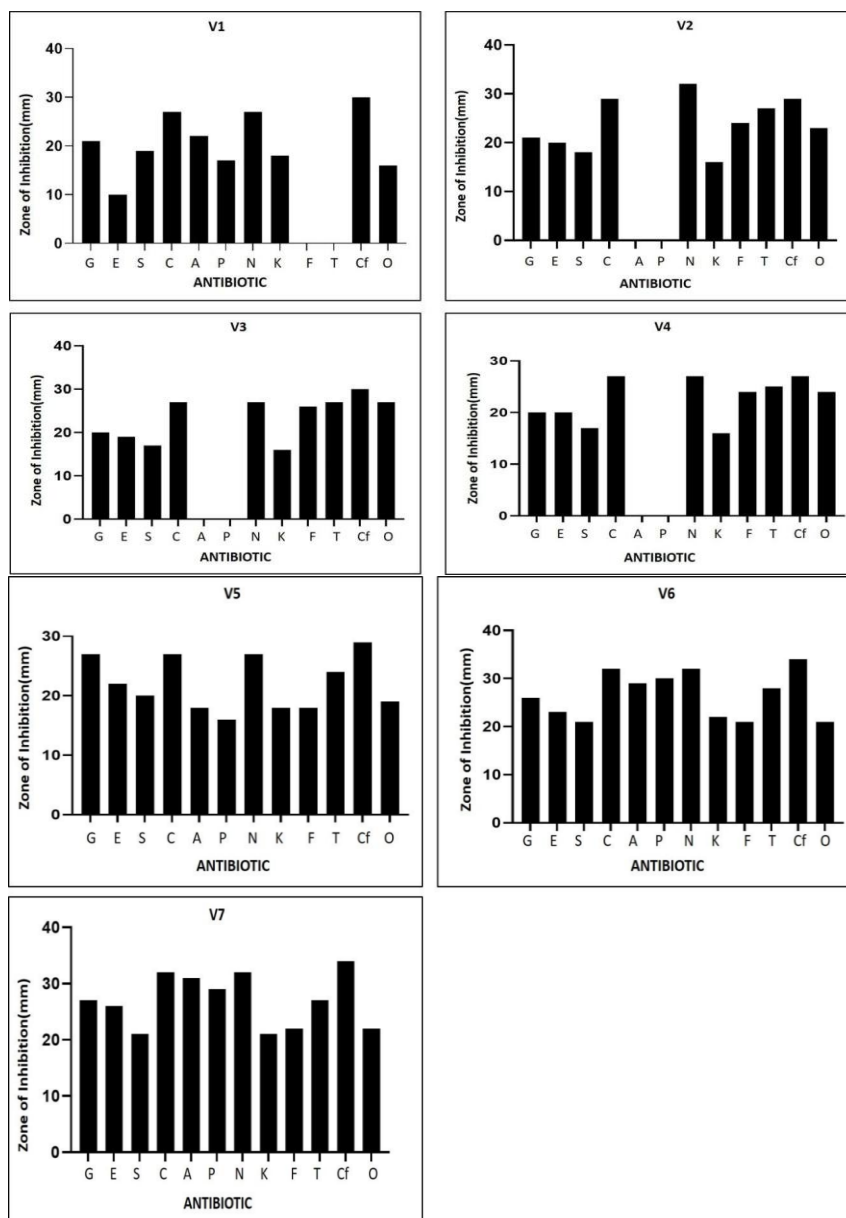


Fig.13. Antibiotic susceptibility test for bacterial isolates

*Gentamycin (G), Erythromycin (E), Streptomycin (S), Chloramphenicol (c), Ampicillin (A), Penicillin-G (P), Norfloxacin (N), Kanamycin (k), Furazolidone (F), Tetracycline (T), Ciprofloxacin (Cf), Oxytetracycline (O).

Discussion

The shrimp industry faces significant challenges from disease outbreaks, particularly White Feces Syndrome (WFS), severely affects *Penaeus vannamei*, studies reported by Jayasree et al. (2006), has shown that *Vibrio* loads in shrimp tissues are significantly higher in diseased individuals. In this study, total bacterial counts (TBC) and total *Vibrio* counts (TVC) in the digestive tract of Normal shrimp were 2×10^5 and 0.43×10^4 CFU/g, respectively, while in White Gut-affected shrimp, they increased to 1.5×10^8 and 1.41×10^7 CFU/g. Similarly, in haemolymph, Normal shrimp showed TBC and TVC of 0.51×10^5 and 0.27×10^5 CFU/ml, whereas affected shrimp exhibited 0.6×10^8 and 0.62×10^6 CFU/ml, respectively. The widespread use of antibiotics in aquaculture has led to antibiotic-resistant bacteria, particularly *Vibrio harveyi*, complicating disease management (Jayasree et al., 2008; Karunasagar et al., 1994). Resistance of *Vibrio* species to Ampicillin and Penicillin-G is a growing concern Defoirdt et al., (2013). In this study, *Vibrio campbellii*, *Vibrio alginolyticus*, and *Shewanella carassii* showed resistance to Penicillin-G, while *Vibrio alginolyticus* and *Shewanella carassii* were also resistant to Ampicillin. *Providencia stuartii* exhibited resistance to Tetracycline and Furazolidone. However, Chloramphenicol was highly effective. whereas, Norfloxacin and Ciprofloxacin remained potent.

Cornejo-Granados et al. (2017) analyzed the microbiota of wild and farmed Pacific White shrimp using 16S rRNA sequencing, highlighting its role in characterizing microorganisms undetectable by traditional methods and the microorganisms that are inadequately distinguished by traditional techniques and offer enhanced characterisation require molecular analysis methods like 16S r-DNA sequencing (Drancourt et al., 2000). In this study, bacterial identification was performed using 16S rRNA sequencing, with phylogenetic analysis based on the Maximum Likelihood method (Kimura, 1980). BLAST analysis of 11 sequences (1173–1521 bp) showed 94.63%–99.86% identity with *Providencia stuartii*, *Vibrio campbellii*, *Vibrio alginolyticus*, *Shewanella carassii*, and *Shewanella* algae. Two strains of *Shewanella carassii* were identified.

Conclusion

These findings emphasize the importance of microbe's identification in shrimp providing a foundation for future research on pathogen management, disease control strategies and the pathogenesis of WFS, ultimately contributing to the development of more efficient and sustainable treatment approaches in shrimp farming.

References

1. APHA. (1992). Standard methods for the examination of water and wastewater (18th ed.). American Public Health Association (APHA), American Water Works Association (AWWA), Water Pollution Control Federation (WPCF), Washington, D.C.
2. Austin, B., & Zhang, X.-H. (2006). *Vibrio harveyi*: A significant pathogen of marine vertebrates and invertebrates. Letters in Applied Microbiology, 43(2), 119–124. <https://doi.org/10.1111/j.1472-765X.2006.01989.x>
3. Baticados, M. C. L., Cruz-Lacierda, E. R., de la Cruz, M. C., Duremdez-Fernandez, R. C., Gacutan, R. Q., Lavilla-Pitogo, C. R., & Lio-Po, G. D. (1990). Diseases of Penaeid Shrimps in the Philippines. Aquaculture Department, SEAFDEC, Tigbauan, Iloilo, Philippines.
4. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1996). Antibiotic susceptibility testing by a standardized single-disc method. American Journal of Clinical Pathology, 45, 493–496.
5. Boyd, C. E., & McNevin, A. A. (2018). Aquaculture, resource use, and the environment. Wiley-Blackwell. <https://doi.org/10.1002/9781118857915>
6. Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environmental Microbiology, 8(7), 1137–1144. <https://doi.org/10.1111/j.1462-2920.2006.01054.x>
7. Chaweepeak, T., Muenthaisong, B., Chaweepeak, S., & Kamei, K. (2015). The potential of galangal (*Alpinia galanga* Linn.) extract against the pathogens that cause white feces syndrome and acute hepatopancreatic necrosis disease (AHPND) in Pacific white shrimp (*Litopenaeus vannamei*). International Journal of Biology, 7(3), 8–17.
8. Chomwong, S., Charoensapsri, W., Amparyup, P., & Tassanakajon, A. (2018). Two host gut-derived lactic acid bacteria activate the proPO system and increase resistance to an AHPND-causing strain of *Vibrio parahaemolyticus* in the shrimp *Litopenaeus vannamei*. Developmental & Comparative Immunology, 89, 54–65.
9. Cornejo-Granados, F., Lopez-Zavala, A. A., Gallardo-Becerra, L., Mendoza-Vargas, A., Sánchez, F., Vichido, R., Brieba, L. G., Viana, M. T., Sotelo-Mundo, R. R., & Ochoa-Leyva, A. (2017). Microbiome of Pacific Whiteleg shrimp reveals differential bacterial community composition between wild, aquacultured, and AHPND/EMS outbreak conditions. Scientific Reports, 7(1), 1–15. <https://doi.org/10.1038/s41598-017-11805-w>
10. Defoirdt, T., Sorgeloos, P., & Bossier, P. (2013). Antibiotic resistance in aquaculture: A growing concern. Aquaculture Research, 44(6), 1–8.
11. Drancourt, M., Bollet, C., Carlouz, A., Martelin, R., Gayral, J. P., et al. (2000). 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. Journal of Clinical Microbiology, 38, 3623–3630.
12. FAO. (2020). The state of world fisheries and aquaculture 2020. Rome: FAO.
13. Felsenstein, J. (1985). Phylogenies and the comparative method. The American Naturalist, 125(1), 1–15. <https://doi.org/10.1086/284325>
14. Flegel, T. W. (2012). The crisis in Asian shrimp aquaculture: Current status and future directions. In Advances in Shrimp Aquaculture (pp. 1–19). CABI Publishing. <https://doi.org/10.1111/j.1439-0426.1998.tb00654.x>
15. Holmström, K., Gräslund, S., Wahlström, A., Pounghompoo, S., Bengtsson, B.-E., & Kautsky, N. (2003). Antibiotic use in shrimp farming and implications for environmental impacts and human health. International Journal of Food

- Science & Technology, 38, 255–266. <https://doi.org/10.1046/j.1365-2621.2003.00671>.
16. Jayasree, L., Janakiram, P., & Madhavi, R. (2006). Characterization of *Vibrio* spp. associated with diseased shrimp from culture ponds of Andhra Pradesh (India). *Journal of the World Aquaculture Society*, 37(4), 523–532.
 17. Janakiram, A., P.V. R. S. R. P. Rao, and A. S. R. Kumar. (2018). "Studies on diseases caused by *Vibrio* spp. in *Penaeus vannamei* from culture ponds of coastal Andhra Pradesh, India." *Aquaculture Research*, 49(1), 259–267. <https://doi.org/10.1111/are.13444>
 18. Jayasree, L., Janakiram, P., & Madhavi, R. (2008). Characterization of bacteria associated with diseased shrimp from culture ponds of Andhra Pradesh (India). *Journal of the World Aquaculture Society*, 39(5), 612–617. <https://doi.org/10.1111/j.1749-7345.2006.00066.x>
 19. Karunasagar, I., K. K. Karunasagar, and J. M. R. K. De. (1994). "Mass mortality of shrimp larvae due to antibiotic-resistant *Vibrio harveyi* in India." *Aquaculture*, 128(3–4), 203–211. [https://doi.org/10.1016/0044-8486\(94\)90289-3](https://doi.org/10.1016/0044-8486(94)90289-3)
 20. Krieg & Holt, 1984. *Bergey's manual of systematic bacteriology*.
 21. Kumar S., Stecher G., and Tamura K. (2015). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* (submitted).
 22. Lightner, D. V. (1988). *Vibrio* diseases of penaeid shrimp. In C. J. Sindermann & D. V. Lightner (Eds.), *Disease Diagnosis and Control in North American Marine Aquaculture* (pp. 42–47). Elsevier.
 23. Lightner, D. V. (1996). *A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp*. Baton Rouge, LA: World Aquaculture Society.
 24. Lightner, D. V. (2011). Virus diseases of farmed shrimp in the Western Hemisphere (the Americas): A review. *Journal of Invertebrate Pathology*, 106, 110–130. <https://doi.org/10.1016/j.jip.2010.09.012>
 25. Li, M., Ghonimy, A., & Chang, Z.-Q. (2024). Profile of the gut microbiota of Pacific white shrimp under industrial indoor farming systems. *Applied Microbiology and Biotechnology*, 108(5), 1234–1245. <https://doi.org/10.1007/s00253-024-13046-0>
 26. Rahman, M. S., & Khan, S. N. (2009). Application of probiotic bacteria: A novel approach towards ensuring food safety in shrimp aquaculture. *Journal of Bangladesh Academy of Sciences*, 33(1), 139–144. <https://doi.org/10.3329/jbas.v33i1.2958>
 27. Roux, K. H. (1995). PCR amplification of GC-rich bacterial 16S rRNA genes. In *Focus*, 17(3), 96–99.
 28. Sriurairatana, S., Boonyawiwat, V., Gangnonngiw, W., Laosutthipong, C., & Hiranchan, J. (2014). White Feces Syndrome of Shrimp arises from transformation, sloughing, and aggregation of hepatopancreatic microvilli into vermiform bodies superficially resembling gregarines. *PLoS ONE*, 9(6), e99170. <https://doi.org/10.1371/journal.pone.0099170>
 29. Somboon, M., Purivirojkul, W., Limsuwan, C., Chuchird, N., 2012. Effect of *Vibrio* spp. in white feces infected shrimp in Chanthaburi, Thailand. *Kasetsart Univ. Fish. Res. Bull.* 36 (1), 7–14.
 30. Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4(4), 406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
 31. Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences*, 101(30), 11030–11035. <https://doi.org/10.1073/pnas.0404206101>
 32. Tang, X., Y. Zhang, and W. Li. (2014). "Microbial community structure in surrounding water of Pacific white shrimp (*Litopenaeus vannamei*) culture ponds." *Journal of Applied Microbiology*, 116(2), 305–313. <https://doi.org/10.1111/jam.12424>
 33. Thompson, F. L., Gomez-Gil, B., Vasconcelos, A. T. R., & Sawabe, T. (2007). Multilocus sequence analysis reveals that *Vibrio harveyi* and *V. campbellii* are distinct species. *Applied and Environmental Microbiology*, 73(13), 4279–4285. DOI: <https://doi.org/10.1128/AEM.00020-07>
 34. Tang, K. F. J., Han, J. E., Aranguren, L. F., White-Noble, B., Schmidt, M. M., Piamsomboon, P., Risdiana, E., & Hanggono, B. (2016). Dense populations of the microsporidian *Enterocytozoon hepatopenaei* (EHP) in feces of *Penaeus vannamei* exhibiting white feces syndrome and pathways of their transmission to healthy shrimp. *Journal of Invertebrate Pathology*, 140, 1–7. <https://doi.org/10.1016/j.jip.2016.08.004>
 35. Tangprasittipap, A., Srisala, J., Chouwddee, S., Somboon, M., Chuchird, N., Limsuwan, C., et al. (2013). The microsporidian *Enterocytozoon hepatopenaei* is not the cause of white feces syndrome in whiteleg shrimp *Penaeus* (*Litopenaeus*) *vannamei*. *BMC Vet. Res.* 9,139. doi: 10.1186/1746-6148-9-139
 36. Wang, H., Wan, X., Xie, G., Dong, X., Wang, X., & Huang, J. (2024). Insights into the histopathology and microbiome of Pacific white shrimp, *Penaeus vannamei*, suffering from white feces syndrome. *Aquaculture Research*, 55(3), 984–997. <https://doi.org/10.1111/are.14527>
 37. Zhang, L., Zhang, Y., & Li, S. (2014b). Molecular diversity of bacteria in the intestine of *Litopenaeus vannamei*. *Journal of Invertebrate Pathology*, 116(1), 44–50. <https://doi.org/10.1016/j.jip.2013.11.003>