Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(3): 1817 – 1837 (2025)
www.ejabf.journals.ekb.eg

Isolation and Characterization of Chitinase-Producing Bacteria in Mangrove Crabs (*Scylla* sp.) with Loose Shell Syndrome

Hans Gabriel M. Gabuat, Ma. Ramela Angela C. Bermeo-Capunong,

Christopher Marlowe A. Caipang*

Division of Biological Sciences, College of Arts and Sciences, University of the Philippines Visayas, Miagao, Iloilo, 5023, Philippines

Corresponding Author: cmacaipang@gmail.com

ARTICLE INFO

Article History:
Received: March 28, 2025
Accepted: May 19, 2025
Online: June 2, 2025

Keywords:
Aquaculture,
Aquatic disease,
Chitinolytic,
Crustacean,
Health management

ABSTRACT

Mangrove crabs (*Scylla* spp.) are ecologically and economically important in coastal regions, particularly in Philippine aquaculture. A novel loose shell syndrome threatens these crab populations, yet its etiology remains unclear. With chitin as essential exoskeletal component in crustaceans, the study aimed to screen, isolate and characterize chitinase-producing bacteria in mangrove crabs affected with loose shell syndrome (LSS). Five mangrove crab samples were procured from the province of Capiz in central Philippines and immediately transported to the laboratory. Homogenized and serially diluted guts, gills, and carapace scrapings were spread-plated onto nutrient agar (NA) media supplemented with 1% NaCl. Subsequently, the isolates were subcultured onto NA plates containing 1% powdered chitin and 1% NaCl to screen for chitinase-producing bacteria. Five isolates showing the highest chitinolytic index values were subjected to further characterization via morphological and biochemical assays, whereas molecular identification was conducted by sequencing the *16S rRNA* gene. Molecular analysis identified the chitinolytic bacteria as belonging to the putative genera *Vibrio*, *Shewanella*, and *Brevundimonas*. The findings of the study suggest a potential link between chitinase-producing bacteria and loose shell syndrome, opening avenues for deeper exploration into its underlying causes and the development of targeted interventions that ensure sustainable aquaculture practices for this commercially important species.

INTRODUCTION

Mangrove crabs (*Scylla* spp.) are members of the Portunidae family and hold major importance in aquaculture due to their widespread distribution **(Macintosh *et al*., 2002; Rahman *et al*., 2020)**. The Philippines boasts a rich heritage in mangrove crab farming, ranking as the world's second-largest producer **(Quinitio, 2017)**. Mangrove crabs display

a remarkable ability to endure extended periods out of water at lower temperatures, which renders them the preferred species for live export to international markets **(Lalramchhani *et al.*, 2019)**. Among the commercially vital species commonly found in the Philippines, *Scylla serrata* is the favored mangrove crab species for cultivation **(Quinitio, 2017)**.

Decapod crustaceans, including mangrove crabs, possess an external integument formed by acellular layers that are primarily composed of chitin and chito-protein matrices strengthened with calcite and an underlying epithelium **(Watling & Thiel, 2013; Zhao *et al.*, 2019)**. Chitin is a homo-polymeric carbohydrate made up of repeating units of N-acetyl-β-D-glucosamine (GIcNAc), functioning as an essential structural framework for the exoskeleton of decapods **(Rowley & Coates, 2023)**.

Certain types of bacteria (*e.g.*, Vibrios and Aeromonads) have been found to detect and identify chitin. These abilities aid in their attachment to the chitinous exoskeleton and the degradation of chitin into saccharides **(Hunt *et al.*, 2008; Aunkham *et al.*, 2018)**. Under normal circumstances and even with the existence of an epibiotic microbial community, significant degradation of crustacean shells is unheard of until they are molted or post-mortem. Nevertheless, there were occurrences of exoskeletal degradation in living crustaceans leading to a disease known as shell disease syndrome—the gradual breakdown of the chitinous exoskeleton via the release of chitinases and various enzymes by microorganisms **(King *et al.*, 2014; Rowley & Coates, 2023)**.

Shell disease in crustaceans, impacting numerous economically relevant species **(Sindermann, 1989)**, is associated with diverse environmental factors **(Noga, 1991)**. Its etiology is multifactorial, primarily involving physical damage to the epicuticle and subsequent chitinolysis mediated by colonizing bacteria **(Cook & Lofton, 1973; Baross *et al.*, 1978; Malloy, 1978)**, and fungi **(Alderman, 1981)**. Contributing environmental elements include elevated nutrient concentrations, water and sediment pollutants, and reduced oxygen levels **(Sindermann, 1989; Noga *et al.*, 2000)**. While typically a superficial condition, severe shell disease can lead to mortality via bacterial infiltration of the hemolymph due to compromised cuticular integrity.

In Capiz, a leading region for mangrove crab aquaculture in the Philippines **(Decembrana, 2017)**, a decline in production has been observed following the emergence of a novel shell disease variant, termed "loose shell syndrome" **(Villarias *et al.*, 2024)**. Local mangrove crab pond operators report atypical symptoms, including the presence of a soft and loose carapace, atrophy of the muscles, and reduced body mass.

Despite the potential harm of loose shell syndrome on the local mangrove aquaculture industry, its etiology is yet to be elucidated in the scientific literature. Therefore, the study aimed to examine the potential role of chitinase-producing bacteria in the disease through screening for chitinolytic activity, isolation, and characterization using morphological, biochemical and *16S rRNA* gene identification. This provided an

improved understanding of the prospective cause of loose shell syndrome in mangrove crabs.

MATERIALS AND METHODS

Sample collection

A total of five (5) diseased mangrove crab samples were collected from a pond in the province of Capiz, the Philippines. The samples were immediately transported live to the University of the Philippines Visayas Microbiology Laboratory. Upon arrival, the crab samples were identified to the species level and dissected, followed by bacterial isolation.

Bacterial isolation and quantification

Bacterial isolates were derived from the carapace scrapings, gills, and gut of the crab samples. Prior to dissection, the carapace was sanitized with 70% alcohol. Then, 500µl of the organ sample was extracted using sterile scissors and forceps, placed in a sterile 1.5ml microcentrifuge tube containing 500µl of 0.9% normal saline solution (NSS). The organ samples were subsequently homogenized.

The homogenized samples were serially diluted from 10^{-1} to 10^{-5}, transferring 100µl to each succeeding tube with 900µl NSS. The tubes were mixed vigorously, ensuring uniform distribution of microbial cells in the organ sample. A 100µl aliquot from every test tube was then sub-cultured aseptically onto nutrient agar (NA) containing 1% NaCl. The organ samples were spread-plated and incubated at 28°C for 24 hours. Standard plate count was performed on each petri dish containing 30-300 colonies. From these isolates, 300 distinct colonies were re-streaked onto fresh NA-1% NaCl plates and incubated at 28°C for 24 hours. All agar plates were stored at 5°C for future analysis.

Screening for chitinolytic activity

To screen for chitin-degrading bacteria, a spot-on-lawn assay was conducted following the methodology of **Whitaker *et al*. (2004)** for the chitin overlay assay with modifications. The initial screening involved inoculating individual colonies on nutrient agar supplemented with 1% colloidal chitin and 1% NaCl (referred to as "chitin agar" hereafter) in triplicates. The plates were then incubated at 28°C for 96 hours. To facilitate the detection and quantification of chitinolytic activity zones around bacterial colonies, the plates were stained with a 0.1% Congo Red solution for 20-30 minutes at room

temperature. Afterward, the plates were destained using a 1% NaCl solution for 5 minutes.

Upon establishing the total number of isolates exhibiting chitinolytic activity, ten (10) isolates with the most extensive chitinolytic activity were chosen to undergo secondary screening of chitinolytic activity at 28°C for 96 hours—each having three replicates. The diameter of the clear zones was then measured in five (5) dimensions due to the irregular nature of the hydrolysis halos using a digital caliper. Mean values of all zone diameter measurements were determined. The chitinolytic index (CI) was computed following the formula of **Korany *et al*. (2019)**.

Isolates with an index of relative enzyme activity value of 1.0 or above are deemed to have considerable enzyme activity **(Duncan *et al*., 2006)**, and they were identified as chitinase-positive isolates. The top five isolates having the highest chitinolytic indices were selected for further characterization. These isolates were individually streaked onto fresh NA-1 % NaCl plates as working cultures and subcultured into NA-1% NaCl slants as stock cultures.

Morphological and biochemical characterization

The chosen isolates were characterized through colony and cellular morphology, chitinase production on chitin agar, and biochemical activities. Morphologically, bacterial colonies were evaluated based on color, form, margin, elevation, opacity, and texture. Cellular features for each isolate were assessed by performing Gram staining and motility test. Biochemical activities examined following the procedures of **Cappuccino and Welsh (2019)** include catalase, casein hydrolysis (protease), lipid hydrolysis (lipase), gelatin hydrolysis (gelatinase), starch hydrolysis (amylase), and hydrogen sulfide production test.

Molecular identification

Genomic bacterial DNA of isolates was extracted using Purelink Genomic DNA Mini extraction kit (Thermo Fisher Scientific) following the manufacturer's procedures. Fresh samples in 5mL nutrient broth cultured overnight were prepared for the DNA extraction. The genomic DNA quantified and stored at -20°C for the subsequent molecular identification.

Polymerase chain reaction (PCR) amplification of *16S rRNA* was carried out following the conditions of **Caipang *et al.* (2010)** and using eubacterial universal primers (Forward: GAGAGTTTGATCCTGGCTCAG and Reverse: CTACGGCTACCTTGTTACGA) in a 25µL PCR reaction. The reaction mixture

consisted of 2µL (10-15ng) of template DNA, 2µL of each primer (5 pmol), 2.5µL of 10X PCR buffer, 1.5µL of 2 mM dNTPs, and 1µL of 50 mM MgCl2, with distilled water added to reach the final volume. The PCR products were purified and sent for sequencing (Macrogen, Korea). The closest homologs of the bacterial isolates were identified by aligning the sequenced data with publicly available sequences in the National Center for Biotechnology Information (NCBI) GenBank database (blast.ncbi.nlm.nih.gov).

Data analysis

Sequences were assembled using BioEdit and were aligned with MAFFT v.7 via the web server **(Katoh & Standley, 2013)**. Following alignment, sequences underwent automated trimming using Phylemon 2.0 (http://phylemon.bioinfo.cipf.es/l). The alignment file was then converted to phylip format using Alignment Transformation Environment or "ALTER" (https://www.sing-group.org/ALTER/) **(Glez-Peña *et al.*, 2010)**. Phylogenetic analysis was performed using the maximum likelihood method by selecting the RAxML program in ALTER. The analysis employed rapid bootstrapping with 1000 replicates, and the resulting phylograms were visualized using FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and were annotated in Microsoft PowerPoint 2024.

RESULTS

Screening for chitinolytic isolates

A total of three hundred (300) bacterial isolates obtained from mangrove crabs with loose shell syndrome were screened for the presence of chitinase activity. The top five isolates that showed the highest chitinolytic activity had chitinolytic indices ranging from 1.17-3.65mm (Fig. 1).

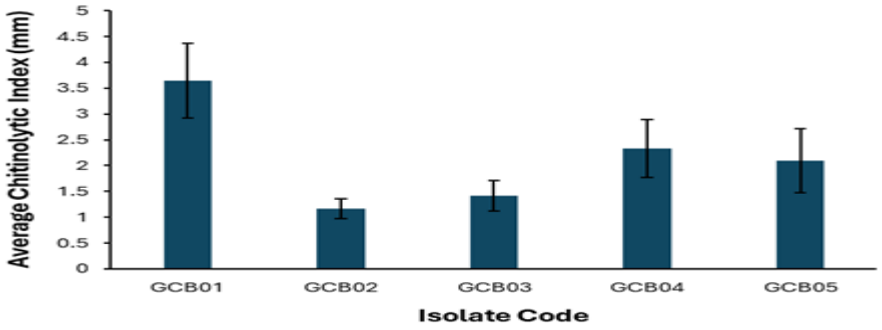

Fig. 1. Average chitinolytic indices of the chitinase-producing bacterial isolates from mangrove crab (*Scylla serrata*)

Morphological and biochemical characterization

Morphological analysis indicated that all isolates are Gram-negative and motile with variations in cell shape: some isolates exhibited a comma shape while others were rod-shaped. All colonies were circular, raised, opaque, and mucoid. Biochemical tests confirmed that all isolates were catalase positive. Table (1) summarizes the morphological, colonial, and biochemical characteristics of each isolate.

Table 1. Morphological, colonial, and biochemical characteristics of the isolates

Morphological, Colonial, and Biochemical Characteristics	GCB01	GCB02	GCB03	GCB04	GCB05
Gram Stain	-	-	-	-	-
Cell Shape	Rod	Rod	Rod	Rod	Rod
Color	White	White	Orange	White	White
Form	Irregular	Irregular	Circular	Irregular	Irregular
Margin	Undulate	Undulate	Entire	Undulate	Undulate
Elevation	Crateriform	Crateriform	Convex	Crateriform	Crateriform
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Texture	Mucoid	Mucoid	Mucoid	Mucoid	Mucoid
Motility	+	+	+	+	+
Amylase	+	+	-	-	-
Lipase	+	+	+	-	-
Protease	-	-	+	-	-
Gelatinase	+	+	+	-	-
Catalase	+	+	+	+	+
Hydrogen Sulfide	-	-	+	-	-

Molecular identification

Table (2) shows the sequence identities of the *16S rRNA* of the chitinolytic isolates with known bacteria obtained from a public database. GCB01 and GCB02 isolates had high sequence identity with *Vibrio*, isolate GCB03 with *Shewanella*, and isolates GCB04 and GCB05 with *Brevundimonas*.

Table 2. Molecular identities of isolated chitinase-producing bacteria by Blastn search via NCBI database

	Scientific Name	Query Coverage (%)	Percent Identity (%)	Accession Number	E-value
GCB01	*Vibrio alginolyticus* strain CIFRI V-TSB1	89%	96%	JF784015.1	0.0
GCB02	*Vibrio natriegens* strain AUCASVE5	96%	97%	JQ277719.1	0.0
GCB03	*Shewanella* sp.	99%	97%	KX254305.1	0.0
GCB04	*Brevundimonas* sp. N5(2011)	86%	98%	JN003651.1	0.0
GCB05	*Brevundimonas diminuta*	99%	95%	KU685402.1	0.0

Phylogenetic analyses showed that isolates GCB01 and GCB02 are closely associated with putative *Vibrio neonatus* (Fig. 2). Isolate GCB03 formed a distinct cluster with *Shewanella algae* (Fig. 3), while isolates GCB04 and GCB05 exhibited close genetic proximity to *Brevundimonas diminuta* (Fig. 4).

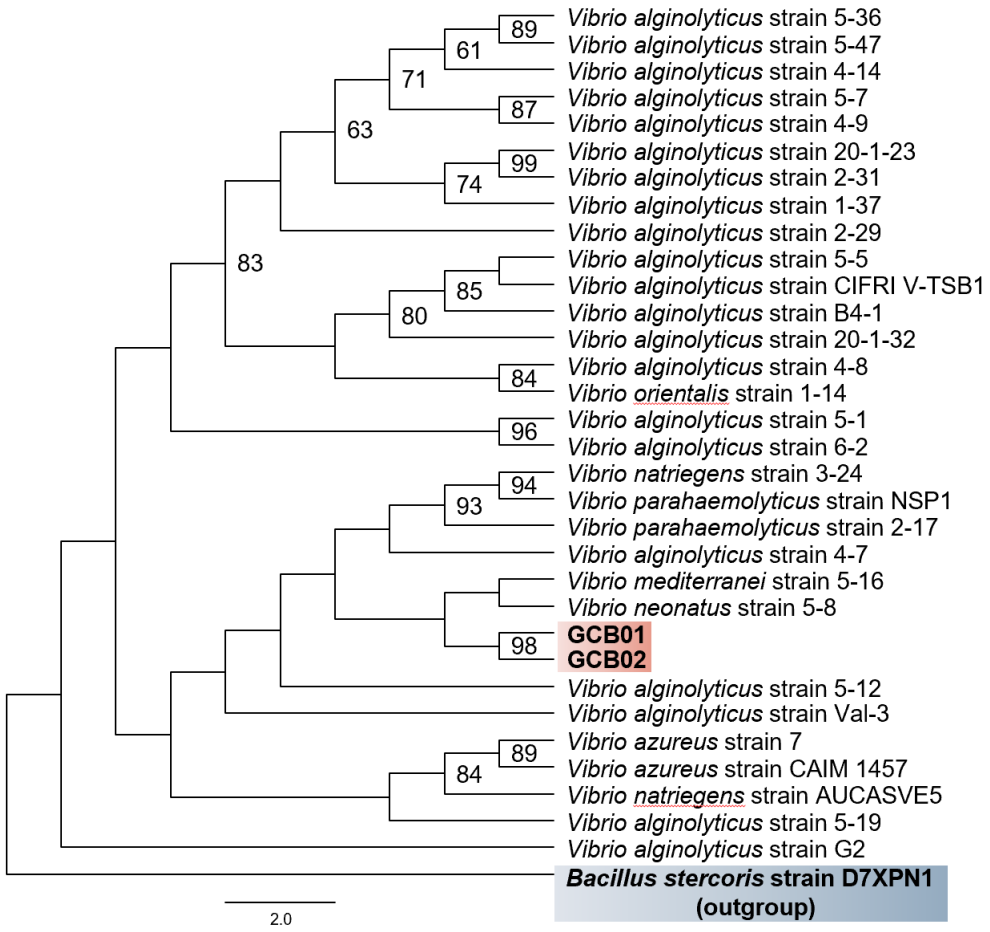

Fig. 2. Maximum likelihood phylogenetic tree of GCB01 and GCB02 isolates and 30 *Vibrio* spp. strains based on partial *16S rRNA* sequences. Values > 60% are shown

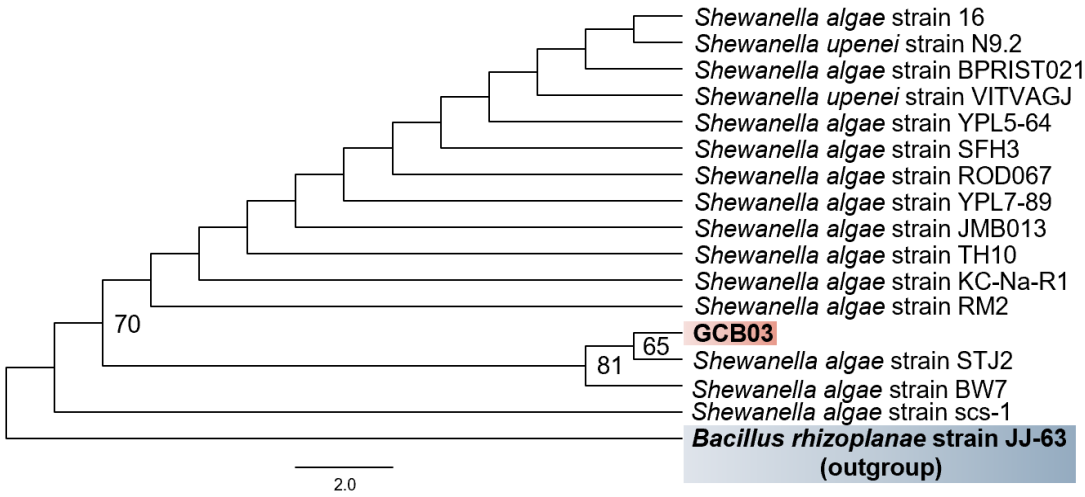

Fig. 3. Maximum likelihood phylogenetic tree of GCB03 isolate and 15 *Shewanella* spp. strains based on partial *16S rRNA* sequences. Values > 60% are shown

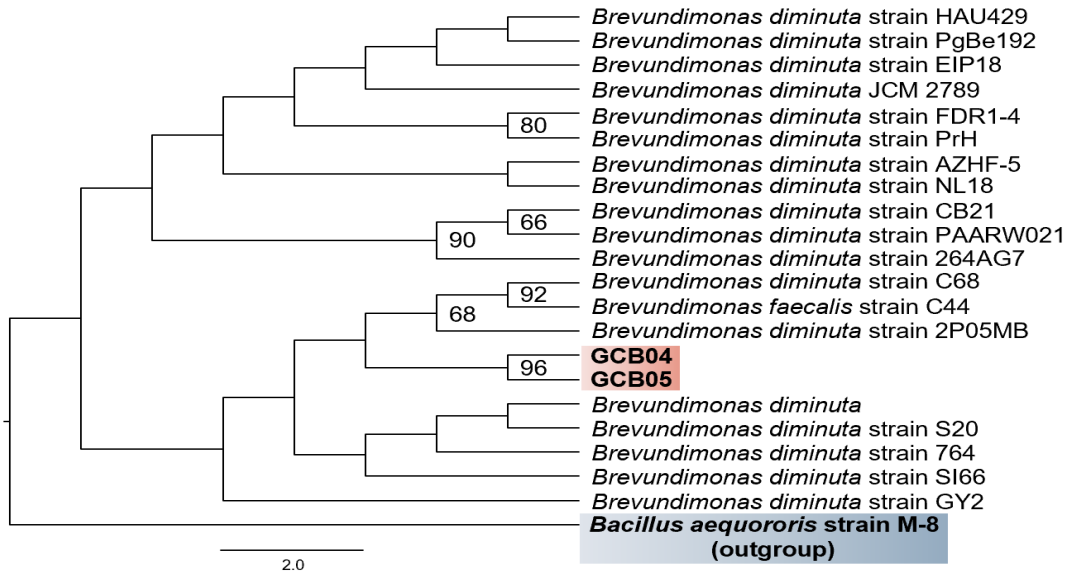

Fig. 4. Maximum likelihood phylogenetic tree of GCB04 and GCB05 isolates and 19 *Brevundimonas* spp. strains based on partial *16S rRNA* sequences. Values > 60% are shown

DISCUSSION

In this study, the isolates underwent both morphological and biochemical characterization. Morphology-wise, every isolate had typical characteristics without any remarkable deviations. However, isolate GCB02, later putatively identified as *Vibrio natriegens*, exhibited pleomorphism unlike the rest, displaying both spherical (coccus) and rod-shaped (bacillus) forms. Although this phenomenon has not been previously reported for the aforementioned species, **Janda *et al*. (1988)** documented that many *Vibrio* spp. are markedly pleomorphic and may exhibit complicated morphology, especially under substandard growth conditions. Representative species include *V. vulnificus* and *V. parahaemolyticus*—the latter can appear in various forms including straight, slightly curved, swollen, and coccoid forms **(Nickelson & Vanderzant, 1971; DiGaetano, 1989)**.

While morphological, biochemical, and other phenotypic characteristics continue to hold importance for genus and species identification, molecular approaches based on nucleotide sequences have demonstrated superior reliability, reproducibility, and robustness **(Durso & Hutkins, 2003)**. The emergence of the "molecular biology age" has introduced a multitude of instruments and techniques for the detection, identification, characterization, and typing of bacteria, serving various clinical and research objectives **(Spratt, 2005)**. A significant portion of contemporary microbial taxonomy and metagenomic analyses rely on investigations of the bacterial *16S rRNA* gene. *16S rRNA* is a constituent of the 30S small subunit of the bacterial ribosome, and it is a vital gene present in all bacteria and archaea **(Hao *et al*., 2017)**. The *16S rRNA* gene is the most widely used housekeeping genetic marker for studying bacterial phylogeny and taxonomy due to several key reasons, summarized in the followings: (1) it is present in nearly all bacteria, often as operons or a multigene family, (2) its function has remained conserved over time, making random sequence changes a reliable measure of evolutionary time, and (3) its length of approximately 1,500 bp is sufficient for informatics analyses **(Janda & Abbott, 2007; Hao *et al*., 2017)**. The standard procedure entails the amplification of the *16S rRNA* gene by PCR, succeeded by sequencing and comparison to established databases to allow for identification **(Franco-Duarte *et al*., 2019)**.

This study employed *16S rRNA* gene sequencing to identify the chitinase-producing isolates. The results revealed that isolates GCB01 and GCB02 were classified within the genus *Vibrio*. Both were identified as putative *Vibrio alginolyticus* strain CIFRI V-TSB1 and *Vibrio natriegens* strain AUCASVE5, respectively. Vibrios are Gram-negative, motile, and comma-shaped bacteria under the phylum Proteobacteria, specifically in the class Gammaproteobacteria, recognized for its diversity among Gram-negative bacteria. Within the order Vibrionales, the family Vibrionaceae includes aquatic bacteria that predominantly inhabit warm waters and can tolerate varying levels of salinity, including

fresh, brackish, and marine environments **(Sampaio *et al.*, 2022)**. **He *et al.* (2020)** report that *Vibrio* spp. have demonstrated the ability to utilize chitin as their sole carbon source, making them crucial chitin-degrading microorganisms in aquatic ecosystems. A prior study determined that 37 out of 47 *Vibrio* strains (80%) possessed the capacity to hydrolyze chitin **(Hunt *et al.*, 2008)**. In addition, **Lin *et al.* (2018)** found that 18 out of 20 *Vibrio* spp. possess genes for chitinase (EC 3.2.1.14) and β-N-acetylhexosaminidase (EC 3.2.1.52)—enzymes which degrade chitin into its monomer form, GlcNAc. Shell disease syndrome is primarily attributed to chitinolytic bacteria, particularly *Vibrio* spp., which act as opportunistic pathogens colonizing the shells of marine crustaceans **(Eddy *et al.*, 2007)**. Although there are no specific records linking *V. alginolyticus* strain CIFRI V-TSB1 to shell disease, *V. alginolyticus* has been frequently isolated from various crustaceans, including different species of crabs, shrimp, and lobsters afflicted with shell disease **(Noga *et al.*, 2000, Vogan *et al.*, 2002; Jayasree *et al.*, 2006; Mancuso *et al.*, 2010, Mancuso *et al.*, 2013)**. For *V. natriegens* strain AUCASVE5, this strain has not been directly associated with shell disease. However, **Bi *et al.* (2016)** found that *V. natriegens* was responsible for the widespread mortalities reported in swimming crabs (*Portunus trituberculatus*) in Jiangsu Province, China. Pathogenic *Vibrio* spp. are known to cause a variety of severe infections and illnesses in humans **(Vezzulli *et al.*, 2013)**. While no available data suggested that the isolates were pathogenic to humans, *V. alginolyticus* is implicated in a range of infections affecting both humans and animals, including ocular and intracranial infections, otitis, osteomyelitis, and peritonitis **(Fu *et al.*, 2016; Jacobs Slifka *et al.*, 2017)**. Moreover, in immunocompromised individuals, it is associated with severe extra-intestinal infections from seafood consumption, leading to fatal diseases like bacteremia, necrotizing soft-tissue infections, multiple organ dysfunction, and septic shock **(Fu *et al.*, 2016)**. On the other hand, despite the extensive use of *V. natriegens* for laboratory work spanning more than 70 years, there are no documented cases of this species causing human infection in the available literature **(Hoff *et al.*, 2020)**.

Isolate GCB03 was identified to be a putative *Shewanella* sp. The genus *Shewanella* is composed of Gram-negative, rod-shaped, oxidase-positive, motile, and facultative anaerobic bacteria **(Thorell *et al.*, 2019; Yu *et al.*, 2022)**. Owing to their remarkable physiological and respiratory adaptability, *Shewanella* spp. thrive in diverse ecological environments, even in extreme conditions such as elevated barometric pressure, high salinity, clinical specimens, and spoiled foods **(Yu *et al.*, 2022)**. The mechanism of pathogenicity of *Shewanella* is not yet fully elucidated. Certain strains of this genus are capable of generating a range of enzymes such as chitinase, protease, lipase, alkylsulfatase, and elastase, potentially contributing to their virulence **(Janda & Abbott, 2014)**. Several species of *Shewanella* have been implicated in shell disease. **Porter *et al.* (2002)** isolated bacterial samples from both healthy and diseased regions of spiny lobsters (*Panulirus argus*), and detected the presence of seven (7) *Shewanella* spp.

In the study of **Chistoserdov *et al*. (2005)**, it was found that lesions in some American lobsters (*Homarus americanus*) with epizootic shell disease were colonized with *S. frigidimarina*. Furthermore, **Bergen *et al*. (2022)** were able to isolate *S. marinintestina* IK-1 and *S. piezotolerans* WP3 from black spot disease lesions in edible crabs (*C. pagurus*). Regarding their pathogenicity to humans, species such as *S. algae*, *S. xiamenensis*, *S. putrefaciens*, and *S. haliotis* have been attributed to cause diseases, including skin and soft tissue infections, peritonitis, pericarditis, bacteremia, bone infections, and pancreatitis **(Janda, 2014; Yu *et al*., 2022)**.

Finally, isolate GCB04 was molecularly identified as putative *Brevundimonas* sp. N5(2011) and isolate GCB05 as a probable *Brevundimonas diminuta*. *Brevundimonas* species are Gram-negative, aerobic, motile, non-fermenting rods, and oxidase and catalase positive, under the Alphaproteobacteria class and Caulobacteraceae family **(Segers *et al*., 1994; Ryan & Pembroke, 2018)**. *Brevundimonas* spp. have been widely detected across numerous environments such as purified water **(Penna *et al*., 2002)**, the condensation water of a Russian space laboratory **(Li *et al*., 2004)** activated sludge **(Ryu *et al*., 2007)**, soils **(Kang *et al*., 2009; Wang *et al*., 2012)**, black sand **(Choi *et al*., 2010)**, various aquatic habitats **(Abraham *et al*., 2010)**, and deep subsea floor sediment **(Tsubouchi *et al*., 2013; Tsubouchi *et al*., 2014)**. Several bacterial species within this genus have also been recognized for their chitinolytic activity **(Jaspers & Overmann, 2004; Warda *et al*., 2016)**. Nevertheless, there are no available data documenting the occurrence of *Brevundimonas* spp. in shell disease. However, *Brevundimonas* spp. were isolated in several species of fish and crustaceans, as part of their microbiota (**Plainpun *et al*., 2011; Ozaktas *et al*., 2012; Parlapani *et al*., 2020)**. There also exist several studies in the scientific literature detailing their pathogenicity in humans. *Brevundimonas* spp. are infrequently held accountable for infections in humans, primarily appearing as nosocomial bacteremia in immunocompromised patients. Among these, *B. diminuta*, *B. nasdae*, and *B. vesicularis* are the only species isolated from human clinical cases, with the remaining 29 species not yet isolated in human hosts **(Lupande-Mwenebitu *et al*., 2021)**. These bacteria are recognized as opportunistic pathogens, causing a range of infections such as meningitis **(Mondello *et al*., 2006)**, endocarditis **(Yang *et al*., 2006; Estrela & Abraham, 2010),** pneumonia/pleuritis **(Donofrio *et al*., 2010)**, keratitis **(Pandit, 2012)**, urinary tract infections **(Shobha *et al*., 2013; Gupta *et al*., 2014)**, and septicemia **(Singh *et al*., 2015)**.

CONCLUSION

This study investigated the potential role of chitinase-producing bacteria in loose shell syndrome affecting mangrove crabs in Capiz, Philippines. The chitinolytic bacterial isolates were identified as *Vibrio*, *Shewanella*, and *Brevundimonas*. This research

represents a significant contribution to understanding the potential microbial factors associated with loose shell syndrome in mangrove crabs from this region. While the study does not definitively establish causality, the isolation of chitinase-producing bacteria from affected crabs warrants further investigation into their role in the development of loose shell syndrome in mangrove crabs. Future research could explore the specific mechanisms by which these bacteria may contribute to shell degradation and the overall health of mangrove crab populations.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Division of Biological Sciences and the National Institute of Molecular Biology and Biotechnology of the University of the Philippines Visayas for providing access to the use of the facilities. This work was partially supported by the DOST-PCAARRD research project, "Molecular Detection of Pathogens in Mangrove Crabs: A Step Towards Ensuring a Sustainable Mangrove Crab Aquaculture Industry".

REFERENCES

Abraham, W.-R.; Estrela, A. B.; Nikitin, D. I.; Smit, J. and Vancanneyt, M. (2010). *Brevundimonas halotolerans* sp. nov., *Brevundimonas poindexterae* sp. nov. and *Brevundimonas staleyi* sp. nov., prosthecate bacteria from aquatic habitats. International Journal of Systematic and Evolutionary Microbiology, 60(8): 1837–1843. https://doi.org/10.1099/ijs.0.016832-0

Alderman, D. J. (1981). *Fusarium solani* causing an exoskeletal pathology in cultured lobsters, *Homarus vulgaris*. Transactions of the British Mycological Society, 76(1): 25–27. https://doi.org/10.1016/S0007-1536(81)80004-6

Aunkham, A.; Zahn, M.; Kesireddy, A.; Pothula, K. R.; Schulte, A.; Baslé, A.; Kleinekathöfer, U.; Suginta, W. and Van Den Berg, B. (2018). Structural basis for chitin acquisition by marine *Vibrio* species. Nature Communications, 9(1): 220. https://doi.org/10.1038/s41467-017-02523-y

Baross, J. A.; Tester, P. A. and Morita, R. Y. (1978). Incidence, microscopy, and etiology of exoskeleton lesions in the Tanner crab, *Chionoecetes tanneri*. Journal of the Fisheries Research Board of Canada, 35(8): 1141–1149. https://doi.org/10.1139/f78-179

Bergen, N.; Krämer, P.; Romberg, J.; Wichels, A.; Gerlach, G. and Brinkhoff, T.

(2022). Shell disease syndrome is associated with reduced and shifted epibacterial diversity on the carapace of the crustacean *Cancer pagurus*. Microbiology Spectrum, 10(6): e03419-22. https://doi.org/10.1128/spectrum.03419-22

Bi, K.; Zhang, X.; Yan, B.; Gao, H.; Gao, X. and Sun, J. (2016). Isolation and molecular identification of *Vibrio natriegens* from diseased *Portunus trituberculatus* in China. Journal of the World Aquaculture Society, 47(6): 854–861. https://doi.org/10.1111/jwas.12305

Caipang, C. M. A.; Brinchmann, M. F. and Kiron, V. (2010). Antagonistic activity of bacterial isolates from intestinal microbiota of Atlantic cod, *Gadus morhua*, and an investigation of their immunomodulatory capabilities. Aquaculture Research, 41(2): 249–256. https://doi.org/10.1111/j.1365-2109.2009.02327.x

Cappuccino, J. G. and Welsh, C. (2019). Microbiology: A Laboratory Manual. 12th ed. New York: Pearson.

Chistoserdov, A. Y.; Smolowitz, R.; Mirasol, F. and Hsu, A. (2005). Culture-dependent characterization of the microbial community associated with epizootic shell disease lesions in American lobster, *Homarus americanus*. Journal of Shellfish Research, 24(3): 741–747. https://doi.org/10.2983/0730-8000(2005)24[741:CCOTMC]2.0.CO;2

Choi, J.-H.; Kim, M.-S.; Roh, S. W. and Bae, J.-W. (2010). *Brevundimonas basaltis* sp. nov., isolated from black sand. International Journal of Systematic and Evolutionary Microbiology, 60(7): 1488–1492. https://doi.org/10.1099/ijs.0.013557-0

Cook, D. W. and Lofton, S. R. (1973). Chitinoclastic bacteria associated with shell disease in *Penaeus* shrimp and the blue crab (*Callinectes sapidus*). Journal of Wildlife Diseases, 9(2): 154–159. https://doi.org/10.7589/0090-3558-9.2.154

Decembrana, A. G. (2017). Mud crab marketing practices. In: Quinitio, E. T.; Parado-Estepa, F. D. and Coloso, R. M., editors. Philippines: In the Forefront of the Mud Crab Industry Development: Proceedings of the 1st National Mud Crab Congress, 16-18 November 2015, Iloilo City, Philippines. Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center. p. 123–126.

DiGaetano, M. (1989). *Vibrio vulnificus* corneal ulcer. Archives of Ophthalmology, 107(3): 323. https://doi.org/10.1001/archopht.1989.01070010333012

Donofrio, R. S.; Bestervelt, L. L.; Saha, R. and Bagley, S. T. (2010). Quantitative real-time PCR and fluorescence in situ hybridization approaches for enumerating *Brevundimonas diminuta* in drinking water. Journal of Industrial Microbiology & Biotechnology, 37(9): 909–918. https://doi.org/10.1007/s10295-010-0738-1

Duncan, S. M.; Farrell, R. L.; Thwaites, J. M.; Held, B. W.; Arenz, B. E.; Jurgens, J. A. and Blanchette, R. A. (2006). Endoglucanase-producing fungi isolated from Cape Evans historic expedition hut on Ross Island, Antarctica. Environmental Microbiology, 8(7): 1212–1219. https://doi.org/10.1111/j.1462-2920.2006.01013.x

Durso, L. and Hutkins, R. (2003). Starter cultures. In: Encyclopedia of Food Sciences and Nutrition. Elsevier. p. 5583–5593.

Eddy, F.; Powell, A.; Gregory, S.; Nunan, L. M.; Lightner, D. V.; Dyson, P. J.; Rowley, A. F. and Shields, R. J. (2007). A novel bacterial disease of the European shore crab, *Carcinus maenas* – molecular pathology and epidemiology. Microbiology, 153(9): 2839–2849. https://doi.org/10.1099/mic.0.2007/008391-0

Estrela, A. B. and Abraham, W.-R. (2010). *Brevundimonas vancanneytii* sp. nov., isolated from blood of a patient with endocarditis. International Journal of Systematic and Evolutionary Microbiology, 60(9): 2129–2134. https://doi.org/10.1099/ijs.0.015651-0

Franco-Duarte, R.; Černáková, L.; Kadam, S.; Kaushik, K. S.; Salehi, B.; Bevilacqua, A.; Corbo, M. R.; Antolak, H.; Dybka-Stępień, K.; Leszczewicz, M. and et al. (2019). Advances in chemical and biological methods to identify microorganisms—From past to present. Microorganisms, 7(5): 130. https://doi.org/10.3390/microorganisms7050130

Fu, K.; Li, J.; Wang, Y.; Liu, J.; Yan, H.; Shi, L. and Zhou, L. (2016). An innovative method for rapid identification and detection of *Vibrio alginolyticus* in different infection models. Frontiers in Microbiology, 7: 651. https://doi.org/10.3389/fmicb.2016.00651

Glez-Peña, D.; Gómez-Blanco, D.; Reboiro-Jato, M.; Fdez-Riverola, F. and Posada, D. (2010). ALTER: program-oriented conversion of DNA and protein alignments. Nucleic Acids Research, 38(suppl_2): W14–W18. https://doi.org/10.1093/nar/gkq321

Gupta, P.; Appannanavar, S.; Kaur, H.; Gupta, V.; Mohan, B. and Taneja, N. (2014). Hospital-acquired urinary tract infection by multidrug-resistant *Brevundimonas vesicularis*. Indian Journal of Pathology and Microbiology, 57(3): 486. https://doi.org/10.4103/0377-4929.138789

Hall, T.; Biosciences, I.; and Carlsbad, C. (2011). BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences, 2(June): 60–61.

Hao, Y.; Pei, Z. and Brown, S. M. (2017). Bioinformatics in microbiome analysis. In: Methods in Microbiology. Vol. 44. Elsevier. p. 1–18.

He, X.; Yu, M.; Wu, Y.; Ran, L.; Liu, W. and Zhang, X.-H. (2020). Two highly

similar chitinases from marine *Vibrio* species have different enzymatic properties. Marine Drugs, 18(3): 139. https://doi.org/10.3390/md18030139

Hoff, J.; Daniel, B.; Stukenberg, D.; Thuronyi, B. W.; Waldminghaus, T. and Fritz, G. (2020). *Vibrio natriegens*: an ultrafast-growing marine bacterium as an emerging synthetic biology chassis. Environmental Microbiology, 22(10): 4394–4408. https://doi.org/10.1111/1462-2920.15128

Hunt, D. E.; Gevers, D.; Vahora, N. M. and Polz, M. F. (2008). Conservation of the chitin utilization pathway in the *Vibrionaceae*. Applied and Environmental Microbiology, 74(1): 44–51. https://doi.org/10.1128/AEM.01412-07

Jacobs Slifka, K. M.; Newton, A. E. and Mahon, B. E. (2017). *Vibrio alginolyticus* infections in the USA, 1988–2012. Epidemiology and Infection, 145(7): 1491–1499. https://doi.org/10.1017/S0950268817000140

Janda, J. M.; Powers, C.; Bryant, R. G. and Abbott, S. L. (1988). Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. Clinical Microbiology Reviews, 1(3): 245–267. https://doi.org/10.1128/CMR.1.3.245

Janda, J. M. and Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of* Clinical Microbiology, 45(9): 2761–2764. https://doi.org/10.1128/JCM.01228-07

Janda, J. M. (2014). *Shewanella*: a marine pathogen as an emerging cause of human disease. Clinical Microbiology Newsletter, 36(4): 25–29. https://doi.org/10.1016/j.clinmicnews.2014.01.006

Janda, J. M. and Abbott, S. L. (2014). The genus *Shewanella*: from the briny depths below to human pathogen. Critical Reviews in Microbiology, 40(4): 293–312. https://doi.org/10.3109/1040841X.2012.726209

Jaspers, E. and Overmann, J. (2004). Ecological significance of microdiversity: identical 16S rRNA gene sequences can be found in bacteria with highly divergent genomes and ecophysiologies. Applied and Environmental Microbiology, 70(8): 4831–4839. https://doi.org/10.1128/AEM.70.8.4831-4839.2004

Jayasree, L.; Janakiram, P. and 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. https://doi.org/10.1111/j.1749-7345.2006.00066.x

Kang, S.-J.; Choi, N.-S.; Choi, J.-H.; Lee, J.-S.; Yoon, J.-H. and Song, J.-J. (2009). *Brevundimonas naejangsanensis* sp. nov., a proteolytic bacterium isolated from soil, and reclassification of *Mycoplana bullata* into the genus *Brevundimonas* as *Brevundimonas bullata* comb. nov. International Journal of Systematic and

Evolutionary Microbiology, 59(12): 3155–3160. https://doi.org/10.1099/ijs.0.011700-0

Katoh, K. and Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution, 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

King, N. G.; Wootton, E. C.; Kennington, K.; Duncan, P. F. and Jenkins, S. R. (2014). Characterisation of shell disease syndrome in the brown crab, *Cancer pagurus*, in a discrete Irish Sea fishery. Journal of Crustacean Biology, 34(1): 40–46. https://doi.org/10.1163/1937240x-00002208

Korany, S. M.; Mansour, A. N.; El-Hendawy, H. H.; Kobisi, A. N. A. and Aly, H. H. (2019). Entomopathogenic efficacy of the chitinolytic bacteria: *Aeromonas hydrophila* isolated from Siwa Oasis, Egypt. Egyptian Journal of Biological Pest Control, 29(1): 16. https://doi.org/10.1186/s41938-019-0116-x

Lalramchhani, C.; Balasubramanian, C. P.; Panantharayil, S. A.; Ghoshal, T. K.; Kumar, P. and Vijayan, K. K. (2019). Mud crab farming: an alternative livelihood in the Indian Sundarban. Indian Journal of Fisheries, 66(2): 56–62.

Li, Y.; Kawamura, Y.; Fujiwara, N.; Naka, T.; Liu, H.; Huang, X.; Kobayashi, K. and Ezaki, T. (2004). *Sphingomonas yabuuchiae* sp. nov. and *Brevundimonas nasdae* sp. nov., isolated from the Russian space laboratory Mir. International Journal of Systematic and Evolutionary Microbiology, 54(3): 819–825. https://doi.org/10.1099/ijs.0.02829-0

Lin, H.; Yu, M.; Wang, X. and Zhang, X.-H. (2018). Comparative genomic analysis reveals the evolution and environmental adaptation strategies of vibrios. BMC Genomics, 19(1): 135. https://doi.org/10.1186/s12864-018-4531-2

Lupande-Mwenebitu, D.; Tshiyongo, R. K.; Lunguya-Metila, O.; Lavigne, J.-P.; Rolain, J.-M. and Diene, S. M. (2021). First isolation and clinical case of *Brevundimonas diminuta* in a newborn with low birth weight, in Democratic Republic of Congo: a case report. Medicina, 57(11): 1227. https://doi.org/10.3390/medicina57111227

Macintosh, D.; Overton, J. L. and Thu, H. V. T. (2002). Confirmation of two common mud crab species (*Genus Scylla*) in the mangrove ecosystem of the Mekong Delta, Vietnam. Journal of Shellfish Research, 21(1): 259–265.

Malloy, S. C. (1978). Bacteria-induced shell disease of lobsters (*Homarus americanus*). Journal of Wildlife Diseases, 14(1): 2–10. https://doi.org/10.7589/0090-3558-14.1.2

Mancuso, M.; Costanzo, M. T.; Maricchiolo, G.; Gristina, M.; Zaccone, R.; Cuccu, D. and Genovese, L. (2010). Characterization of chitinolytic bacteria and histological aspects of Shell Disease Syndrome in European spiny lobsters

(*Palinurus elephas*) (Fabricius 1787). Journal of Invertebrate Pathology, 104(3): 242–244. https://doi.org/10.1016/j.jip.2010.03.009

Mancuso, M.; Zaccone, R.; Carella, F.; Maiolino, P. and De Vico, G. (2013). First episode of shell disease syndrome in *Carcinus aestuarii* (Crustacea: Decapoda: Portunidae) in the Volturno River. Journal of Aquaculture Research & Development, 4(5). https://doi.org/10.4172/2155-9546.1000191

Mondello, P.; Ferrari, L. and Carnevale, G. (2006). Nosocomial *Brevundimonas vesicularis* meningitis. Infezioni in Medicina, 14: 235–237.

Nickelson, R. and Vanderzant, C. (1971). *Vibrio parahaemolyticus* – A review. Journal of Milk and Food Technology, 34(9): 447–452. https://doi.org/10.4315/0022-2747-34.9.447

Noga, E. J. (1991). Shell disease in marine crustaceans: concluding remarks. Journal of Shellfish Research, 10: 505–506.

Noga, E. J.; Smolowitz, R. and Khoo, L. H. (2000). Pathology of shell disease in the blue crab, *Callinectes sapidus* Rathbun (Decapoda: Portunidae). Journal of Fish Diseases, 23(6): 389–399. https://doi.org/10.1046/j.1365-2761.2000.00249.x

Ozaktas, T.; Taskin, B. and Gozen, A. G. (2012). High-level multiple antibiotic resistance among fish surface-associated bacterial populations in non-aquaculture freshwater environments. Water Research, 46(19): 6382–6390. https://doi.org/10.1016/j.watres.2012.09.010

Pandit, R. T. (2012). *Brevundimonas diminuta* keratitis. Eye & Contact Lens: Science and Clinical Practice, 38(1): 63–65. https://doi.org/10.1097/ICL.0b013e31821c04f7

Parlapani, F. F.; Ferrocino, I.; Michailidou, S.; Argiriou, A.; Haroutounian, S. A.; Kokokiris, L.; Rantsiou, K. and Boziaris, I. S. (2020). Microbiota and volatilome profile of fresh and chill-stored deepwater rose shrimp (*Parapenaeus longirostris*). Food Research International, 132: 109057. https://doi.org/10.1016/j.foodres.2020.109057

Penna, V. T. C.; Martins, S. A. M. and Mazzola, P. G. (2002). Identification of bacteria in drinking and purified water during the monitoring of a typical water purification system. BMC Public Health, 2(1): 13. https://doi.org/10.1186/1471-2458-2-13

Plainpun, N.; Tunkijjanukij, S.; Kankamo, C. and Salaenoi, J. (2011). Identification of bacterial strains in red thoracic-abdominal syndrome. Paper presented at: The 3rd International Conference on Sustainable Animal Agriculture for Developing Countries (SAADC2011); July 26-29, Nakhon Ratchasima, Thailand.

Porter, L.; Butler IV, M. and Reeves, R. H. (2002). Normal bacterial flora of the spiny lobster *Panulirus argus* and its possible role in shell disease. Marine and Freshwater

Research, 52(8): 1401. https://doi.org/10.1071/MF01092

Quinitio, E. T. (2017). Overview of the mud crab industry in the Philippines: In the forefront of the mud crab industry development (pp. 1-12). In Proceedings of the 1st National Mud Crab Congress, Iloilo City, Philippines. Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Rahman, M. M.; Haque, S. M.; Islam, M. A.; Paul, A. K.; Iqbal, S.; Atique, U.; Wahab, A.; Egna, H. and Brown, C. (2020). Assessment of mud crab fattening and culture practices in coastal Bangladesh: Understanding the current technologies and development perspectives. AACL Bioflux, 13(2): 582-596.

Rowley, A. F. and Coates, C. J. (2023). Shell disease syndromes of decapod crustaceans. Environmental Microbiology, 25(5): 931–947. https://doi.org/10.1111/1462-2920.16344

Ryan, M. P. and Pembroke, J. T. (2018). *Brevundimonas* spp: Emerging global opportunistic pathogens. Virulence, 9(1): 480–493. https://doi.org/10.1080/21505594.2017.1419116

Ryu, S. H.; Park, M.; Lee, J. R.; Yun, P. Y. and Jeon, C. O. (2007). *Brevundimonas aveniformis* sp. nov., a stalked species isolated from activated sludge. International Journal of Systematic and Evolutionary Microbiology, 57(7): 1561–1565. https://doi.org/10.1099/ijs.0.64737-0

Sampaio, A.; Silva, V.; Poeta, P. and Aonofriesei, F. (2022). *Vibrio* spp.: Life strategies, ecology, and risks in a changing environment. Diversity, 14(2): 97. https://doi.org/10.3390/d14020097

Segers, P.; Vancanneyt, M.; Pot, B.; Torck, U.; Hoste, B.; Dewettinck, D.; Falsen, E.; Kersters, K. and De Vos, P. (1994). Classification of *Pseudomonas diminuta* Leifson and Hugh 1954 and *Pseudomonas vesicularis* Busing, Doll, and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov., respectively. International Journal of Systematic Bacteriology, 44(3): 499–510. https://doi.org/10.1099/00207713-44-3-499

Shobha, K. L.; Ramachandra, L.; Gowrish, S. and Nagalakshmi, N. (2013). *Brevundimonas diminuta* causing urinary tract infection. WebmedCentral, 4: WMC004411.

Sindermann, C. J. and Northeast Fisheries Center. (1989). The shell disease syndrome in marine crustaceans. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Center.

Singh, S.; Bhatia, B. D.; Ansary, A.; Gambhir, P.; Anand, M. R.; Parakh, M.;

Agarwal, S.; Roy, M. P.; Walsh, K.; John, T. J. and Pothapregada, S. (2015). Correspondence. Indian Pediatrics, *52*(10), 901–907. https://doi.org/10.1007/s13312-015-0743-6

Spratt, D. A. (2005). Significance of bacterial identification by molecular biology methods. Endodontic Topics, 9(1): 5–14. https://doi.org/10.1111/j.1601-1546.2004.00106.x

Thorell, K.; Meier-Kolthoff, J.P.; Sjöling, Å. and Martín-Rodríguez, A.J. (2019). Whole-genome sequencing redefines *Shewanella* taxonomy. Frontiers in Microbiology, 10:1861. https://doi.org/10.3389/fmicb.2019.01861

Tsubouchi, T.; Shimane, Y.; Usui, K.; Shimamura, S.; Mori, K.; Hiraki, T.; Tame, A.; Uematsu, K.; Maruyama, T. and Hatada, Y. (2013). *Brevundimonas abyssalis* sp. nov., a dimorphic prosthecate bacterium isolated from deep-subsea floor sediment. International Journal of Systematic and Evolutionary Microbiology, 63(Pt 6):1987–1994. https://doi.org/10.1099/ijs.0.043364-0

Tsubouchi, T.; Koyama, S.; Mori, K.; Shimane, Y.; Usui, K.; Tokuda, M.; Tame, A.; Uematsu, K.; Maruyama, T. and Hatada, Y. (2014). *Brevundimonas denitrificans* sp. nov., a denitrifying bacterium isolated from deep subseafloor sediment. International Journal of Systematic and Evolutionary Microbiology, 64(Pt 11):3709–3716. https://doi.org/10.1099/ijs.0.067199-0

Vezzulli, L.; Colwell, R.R. and Pruzzo, C. (2013). Ocean warming and spread of pathogenic *Vibrios* in the aquatic environment. Microbial Ecology, 65(4):817–825. https://doi.org/10.1007/s00248-012-0163-2

Villarias, M.P.; Gabuat, H.G.; Romey, M.T.; Pakingking, R.; Philip, G.; Fagutao, F.F.; Suharman, I. and Caipang, C.M. (2024). Antibiotic resistance and molecular identification of dominant bacteria associated with loose shell syndrome in mangrove crabs (*Scylla* spp.). BIO Web of Conferences, 136:05004. https://doi.org/10.1051/bioconf/202413605004

Vogan, C.L.; Costa-Ramos, C. and Rowley, A.F. (2002). Shell disease syndrome in the edible crab, *Cancer pagurus* – isolation, characterization and pathogenicity of chitinolytic bacteria. Microbiology, 148(3):743–754. https://doi.org/10.1099/00221287-148-3-743

Wang, J.; Zhang, J.; Ding, K.; Xin, Y. and Pang, H. (2012). *Brevundimonas viscosa* sp. nov., isolated from saline soil. International Journal of Systematic and Evolutionary Microbiology, 62(Pt 10):2475–2479. https://doi.org/10.1099/ijs.0.035352-0

Warda, E.A.; Abeer, A.A.E.A.; Eman, R.H.; Mahmoud, A.S. and Ahmed, I.E.-D. (2016). Applications of Plackett–Burman and central composite design for the optimization of novel *Brevundimonas diminuta* KT277492 chitinase production,

investigation of its antifungal activity. Brazilian Archives of Biology and Technology, 59:0. https://doi.org/10.1590/1678-4324-2016160245

Watling, L. and Thiel, M. (2013). *Functional Morphology and Diversity*. Oxford University Press.

Whitaker, J.O.; Dannelly, H.K. and Prentice, D.A. (2004). Chitinase in insectivorous bats. Journal of Mammalogy, 85(1):15–18. https://doi.org/10.1644/1545-1542(2004)085<0015: CIIB>2.0.CO;2

Yang, M.-L.; Chen, Y.-H.; Chen, T.-C.; Lin, W.-R.; Lin, C.-Y. and Lu, P.-L. (2006). Case report: Infective endocarditis caused by *Brevundimonas vesicularis*. BMC Infectious Diseases, 6(1):179. https://doi.org/10.1186/1471-2334-6-179

Yu, K.; Huang, Z.; Xiao, Y. and Wang, D. (2022). *Shewanella* infection in humans: Epidemiology, clinical features and pathogenicity. Virulence, 13(1):1515–1532. https://doi.org/10.1080/21505594.2022.2117831

Zhao, X.; Zhang, J. and Zhu, K.Y. (2019). Chito-protein matrices in arthropod exoskeletons and peritrophic matrices. In: Cohen, E. and Merzendorfer, H. (Eds.), Extracellular Sugar-Based Biopolymer Matrices, Vol. 12. Springer International Publishing, Cham. pp. 3–56. https://doi.org/10.1007/978-3-030-12919-4_1