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Response of *Macrobrachium rosenbergii* juvenile against a virulent isolate of *Vibrio* sp. from a diseased *Penaeus monodon*

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Abstract. The present study investigated the *in vivo* virulence of a *Vibrio* sp. isolated from a diseased tiger shrimp (*Penaeus monodon*) and determined the median lethal concentration (LC_{50}) of *Vibrio* sp. against giant prawn (*Macrobrachium rosenbergii*) juveniles at the cell densities of 10^4, 10^5, 10^6, 10^7 CFU/mL. In addition, the survival rate of *M. rosenbergii* was examined through a bath challenge test against the *Vibrio* sp. isolate at different cell densities. The results showed that the median LC_{50} of *Vibrio* sp. for *M. rosenbergii* was 10^6 CFU/mL by probit-transformed responses. *Vibrio* sp. concentrations had no effect on the mortality of prawn juveniles; however, infection time might significantly increase the mortality (p = 0.000). Lower survival of prawn juveniles was also recorded at the bacterial cell density of 10^6 CFU/mL. Finally, *Vibrio* sp. cell density 10^4 CFU/mL showed lower cumulative percent survival and higher hazard function compared to other concentrations. This study provides an indication that pathogenic Vibrios of tiger shrimp can be a potential risk for *M. rosenbergii* larvae culture. Further studies should be conducted to unravel the mechanism of actual mortality factor, possibly quorum sensing regulatory activities of the *Vibrio* sp. isolate.
Keywords: *Macrobrachium rosenbergii*, *Vibrio* sp., *Penaeus monodon*, LC_{50}

Introduction

Giant freshwater prawn *Macrobrachium rosenbergii* contributes 24% of total 396 million USD export in come of Bangladesh (DoF 2018). Due to the increased demand of prawns in the international market, prawn hatcheries are now being extended worldwide, attracting many fish farmers to prawn cultivation. However, mass mortality of prawn in the hatchery and grow-out culture is a concern for the growth of this industry. The mortality caused primarily due to the attack of bacteria, viruses, protozoa, and fungi (Bonami and Widada 2011, Siripornadulsil et al. 2014, Mandal et al. 2015, de Souza Valente and Wan 2021, Hooper et al. 2023).

Outbreak of diseases in prawn farms are often attributed to bacterial infections (Phatarpekar et al. 2002, Sung et al. 2000), including the species under the genus *Vibrio* (*Vibrio harveyi, V. anguillarum, V. parahaemolyticus* and *V. vulnificus*), *Leucothrix*, *Enterobacter* or *Staphylococcus* sp. (Jayasree et al. 2006, Briggs 2013, Mastan and Begum 2016, de Souza Valente and Wan 2021). Vibriosis is one of the most devastating problems for penaeid and non-penaeid shrimp juveniles (Ninawe and Selvin 2009). Previous studies described several *Vibrio* spp., including *V. cholerae*, *V. harveyi* and *V. parahaemolyticus* pathogenic towards the larvae of *M. rosenbergii* and *Penaeus monodon* (Joseph et al. 2015, Amar et al. 2022, Hooper et al. 2023). Giant freshwater prawn and tiger shrimp share same ecosystem in the early life stages, and polyculture of these two species becoming popular in the coastal aquaculture ponds (Mamun 2020, Barua and Rahman 2020, Jewel et al. 2021, Mondal et al. 2021). Therefore, it is highly likely to

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transmit pathogens between species sharing ecosystems and become virulent by several virulence regulatory mechanisms through quorum sensing signal pathways within suitable environmental windows (Bruto et al. 2017, Hooper et al. 2023, Islam et al. 2022).

Understanding the virulence of a pathogen, determination of 50% mortality of experimental animals (LC_{50} value) is a common practice in fish and shellfish disease study (Hodgson and Rose 2008, Kuçukgul Gulec et al. 2013, Wang et al. 2015, Jiang et al. 2019). On the other hand, infection study of virulent vibrios through in vivo challenge tests are conducted to determine the virulence potential towards *M. rosenbergii* and other shellfishes (Lavilla-Pitogo et al. 1990, Soto-Rodríguez et al. 2006, Phuoc et al. 2008, Sung et al. 2008, Wang et al. 2015, Azad et al. 2019). Despite extensive research worldwide, there has been a little study on the pathogenicity of *Vibrio* spp. and their associated vibriosis diseases on the prawn aquaculture in Bangladesh. Production and economic losses in *Macrobrachium* aquaculture, either due to early mortality at the hatchery and nursery stages or at the grow-out phase are not well studied in Bangladesh. Therefore, study of virulent bacterial isolates, including their virulence mechanisms, disease transfer between hosts and pathogenicity should be determined to understand the complex disease mechanisms of vibrios. This study aimed to examine the median lethal concentration (LC_{50}) of a *Vibrio* sp. isolated from a diseased *Penaeus monodon* towards *M. rosenbergii* juvenile. This study also investigated the survival of *M. rosenbergii* juvenile against the challenge of isolated *Vibrio* sp. This study discusses that virulent strain of *Vibrio* sp. isolated from *P. monodon* can be pathogenic towards *M. rosenbergii*. This study also claims the median lethal concentration of the isolate and predicts the probability of cumulative survival and hazard function of the isolate at different cell densities.

Materials and Methods

Study area and period: The experiment was conducted in the Water Chemistry Lab, Quality Control Laboratory and Fish Biochemistry and Molecular Genetics Laboratory of Fisheries and Marine Resource Technology (FMRT) Discipline, Khulna University, Bangladesh. Fieldwork, laboratory work and challenge tests were conducted from May to July. Bacterial strain was collected from the laboratory stock of Shrimp Research Station, Bangladesh Fisheries Research Institute (BFRI), Bagerhat.

Bacterial strain collection and maintenance: Pure culture of bacterial strain was isolated from a diseased tiger shrimp. Diseased shrimps were dissected, gut and intestine pooled, 1 g of the pooled samples homogenized with 1 ml of sterilized source water (from where samples were collected) and transferred to a 2 ml microtube. 100 µl of sample solution was spread on Thio-sulphate Citrate Bile Salt Sucrose (TCBS) Agar plate for selective isolation of *Vibrio* spp., and incubated at room temperature for 24-48 h. From the dominant colonies, one single green to blue colony of 2-3 mm was picked with a sterile tooth pick and streaked on a new TCBS agar plate, followed by three consecutive cultures from single colonies on TCBS agar plate to isolate the pure colony.

The bacterial culture was maintained in LB_{10} broth, prepared by dissolving 10 g tryptone (Biokar diagnostic), 5 g of yeast extract (Biokar diagnostic) and 10 g Instant Ocean in 1 L demineralized water, followed by autoclaving at 121°C for 20 min. The culture tubes were incubated at 30°C on a shaker for 24 h. For long term storage, the bacterial culture (absorbance 1.0, OD 600) was stored in 40% autoclaved glycerol at -80°C. Freshly cultured bacteria from stock solution (1% in freshly prepared LB_{10} medium) were used for *in vivo* challenge experiment. The isolate was found virulent in *in-vitro* swimming motility and casein enzyme degradation assay (our unpublished data). The pathogenicity test experiment confirmed that this isolate can infect *M. rosenbergii* juveniles with some symptoms, including tail erosion, white spots on carapace, and pale colouration of whole body (our unpublished data).

Experimental layout: This study was conducted to determine the LC_{50} and survival of *M. rosenbergii* at different concentrations of *Vibrio* sp. through a bath challenge test. The bacterial concentrations were 10^4, 10^5, 10^6, 10^7 CFU/mL with a control group (C) without *Vibrio* sp. All these bacterial concentrations were designated as the treatments (e.g., such as T1, T2, T3 and T4, respectively) with three replicates per treatment to check the reproducibility. The challenge test was carried out in 30 litter plastic tanks containing 25 L water. All the tanks were stocked with 10 juvenile prawns of 2 - 3 g. Post-larvae (PL_{45}) was collected from BFRI, Bagerhat, nursed for 60 days and used in this experiment(Azad et al., 2023). The juveniles were acclimatized for five days before inoculating the bacteria. The pathogenic bacterial isolate was cultured overnight in LB_{10} medium, bacterial density observed (absorbance 1.0, OD 600) by spectrophotometer (T60 UV-VIS Spectrophotometer) and estimated cell density nearly at 7.8×10^8 CFU/mL (OD 1.0 ~ 7.8×10^8 CFU/mL) (Volkmer and Heinemann 2011). The pathogen was added in the culture tank at a final density of 10^4, 10^5, 10^6, 10^7 CFU/mL. During the challenge test, the juveniles were fed with Mega prawn grower feed (Code. 404, pellet diameter = 2 mm) with 32% protein at 10% of their body weight, and feeding was done 2-3 times daily. After pathogenic infection, the feeding rate was reduced to 5% of the body weight to reduce organic load in the system and lower the experimental contamination.

The salinity of the tank water was adjusted to 10 ppt with 120 ppt brine water. Other environmental parameters like dissolve oxygen, temperature and pH were maintained at a constant level. Dissolve oxygen was maintained by using constant aeration with same flow rate in each tank to maintain the DO level of 4-5 mg/L. Water temperature was controlled and maintained 30°C throughout the experiment by an aquarium heater (hygger, HG-802, IC Aquarium Heater, Shenzhen, China). Water pH was measured daily by using HACH kit, produced by HACH, USA (Model FF-2) and found within suitable range (7.5-8.0) for prawn culture, and water pH was adjusted with NaOH solution if required (Azad et al., 2021). Only 5-10% water was adjusted daily to compensate the loss through evaporation and siphoning. The bacterial load of each treatment was checked daily on TCBS agar plate and enumerated by standard plate count method (Islam, 2016). The experiment was continued for 30 days, and the subsequent mortality was recorded throughout the experiment.

Data analysis: The LC_{50} was determined by probit regression analysis using SPSS 23.0. Time difference for 50% mortality among the treatment was determined by one-way ANOVA. Effect of different bacterial concentrations on the mortality of prawn juveniles was determined by Poisson Regression and survival analysis was done by Independent-Samples Kruskal-Wallis Test. The hazard functions were determined through Kapalan Meier survival test.

Results

The results of this work presented the percent mortality of giant freshwater prawn *M. rosenbergii* when exposed to four different concentrations (10^4, 10^5, 10^6, 10^7 CFU/mL) of pathogenic *Vibrio* sp. The mortality of juvenile was only 20% in the control group; hence this result was not presented in LC_{50} determination.

Determination of LC_{50}

There was no significant difference between the bacterial cell concentrations on the 50% mortality of prawn juveniles. The lowest time for 50% mortality was observed at the bacterial density of 10^6 CFU/mL, followed by 10^4, 10^7 and 10^5 CFU/ mL (Fig. 1). The probit regression analysis also indicated 10^6 CFU/mL as the LC_{50} (Fig. 2).

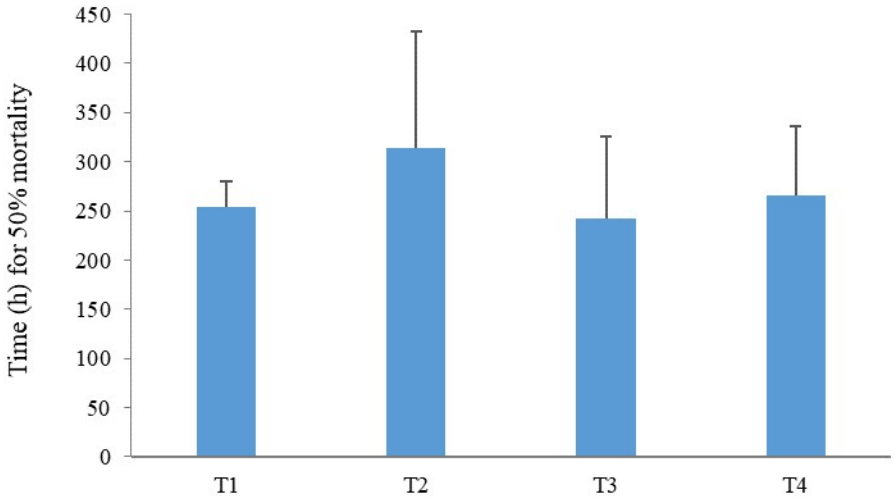

Fig. 1. Time required for 50% mortality of prawn juveniles challenged with *Vibrio* sp. at different cell densities. The error bars represent the standard deviations of three replicates.

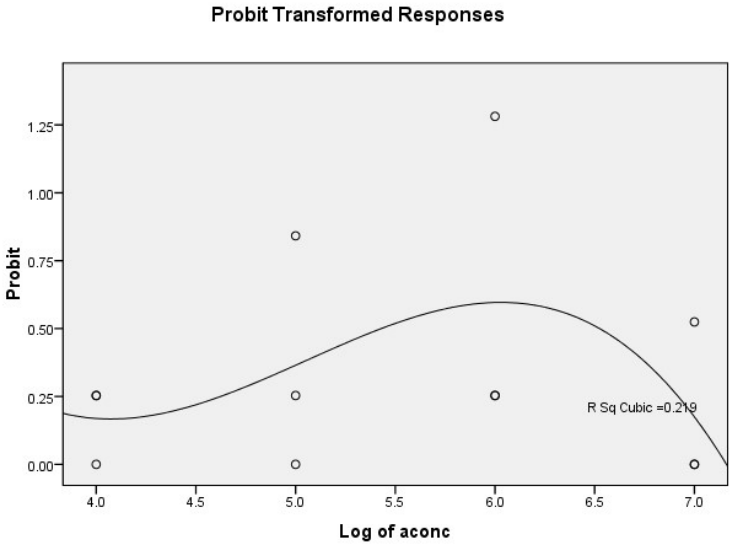

Fig. 2. The graph shows percent of mortality and probit mortality of the prawn juvenile.

Effect of *Vibrio* sp. concentrations on prawn mortality

The test of model effects demonstrated that different concentrations of *Vibrio* sp. used in this study did not have any significant effect on the mortality of prawn juveniles (P = 0.867). On the other hand, exposure time had a significant effect on the juvenile mortality (P = 0.000) (Tables I and II). That means exposure of prawn juveniles against *Vibrio* sp. for a long time might be responsible for their higher mortality.

Table I. Tests of Model Effects on the mortality of prawn juvenile against *Vibrio* sp.

	Type III		
Source	Wald Chi-Square	df	Sig.
(Intercept)	193.300	1	.000
Treatment	0.726	3	.867
Time	14.180	1	.000

Dependent Variable: Mortality
Model: (Intercept), Treatment, Time

Table II. Parameter estimates on the mortality of prawn juvenile against *Vibrio* sp.

			95% Wald Confidence	
Parameters	B	Std. Error	Interval	Hypothesis Test

			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-4.896	0.4061	-5.692	-4.100	145.38	1	0.00
[Treatment=1.00]	3.609E-18	0.3430	-0.672	0.672	0.00	1	1.00
[Treatment=2.00]	0.111	0.3338	-0.543	0.766	0.111	1	0.739
[Treatment=3.00]	0.235	0.3263	-0.404	0.875	0.521	1	0.471
[Treatment=4.00]	0[a]	.	.	.	.	.	.
Time	0.137	0.0363	0.066	0.208	14.18	1	0.00
Scale	1[b]						

Dependent Variable: Mortality Model:
(Intercept), Treatment, Time

a. Set to zero because this parameter is redundant.

b. Fixed at the displayed value.

Survival of *M. rosenbergii* against *Vibrio* sp.

Survival of the prawn juvenile over a 30-day challenge test with *Vibrio* sp. showed that this bacterium was highly pathogenic to prawns as only 30±17.32% survival was found at a concentration of 10^6 CFU/mL regardless of non-significant. Similarly, lower survival was also observed at the concentration of 10^5 CFU/ mL (33.33±11.55%) compared to 10^4 CFU/ mL (43.33±5.77%) and 10^7 CFU/ mL (43.33±11.55%) (Fig. 4).

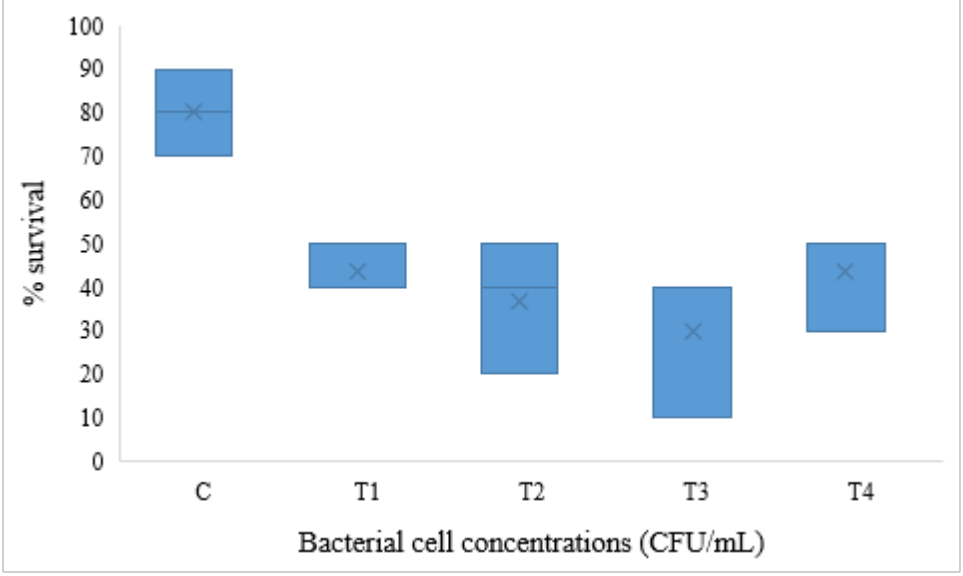

Fig. 4. Survival of *M. rosenbergii* challenged with pathogenic *Vibrio* sp. There were no significant differences between the treatments (Log Rank (Mantel-Cox) pairwise comparison, $P < 0.05$).

Survival function

Within the 30-day challenge test, the probability of cumulative survival of *M. rosenbergii* in the control group and T3 were ≥ 80% up till the end of the experiment. Probability of cumulative survival was close to 80% in T1 at the middle of the experiment (15-16 days), which then felt suddenly to 50% within the next two days. T2 and T4 had 80% survival function at 250 and 350 h, respectively, which then reduced to less than 70% and continued up till the end of the experiment (Fig. 5).

Hazard function

The probability of cumulative hazard was higher in T1, followed by T2 and T4. Bacterial cell concentration of 10^4 CFU/mL was the most hazardous to *M. rosenbergii*. During the first 300 to 400 hrs, 10^4 CFU/mL was found less hazardous than other treatments. However, suddenly after 400 hrs, there was two steps boosting of the probability of cumulative hazard at 10^4 CFU/mL, which made it more hazardous (Fig. 6). Thus, time function is considerable in the mortality of *M. rosenbergii* against *Vibrio* sp.

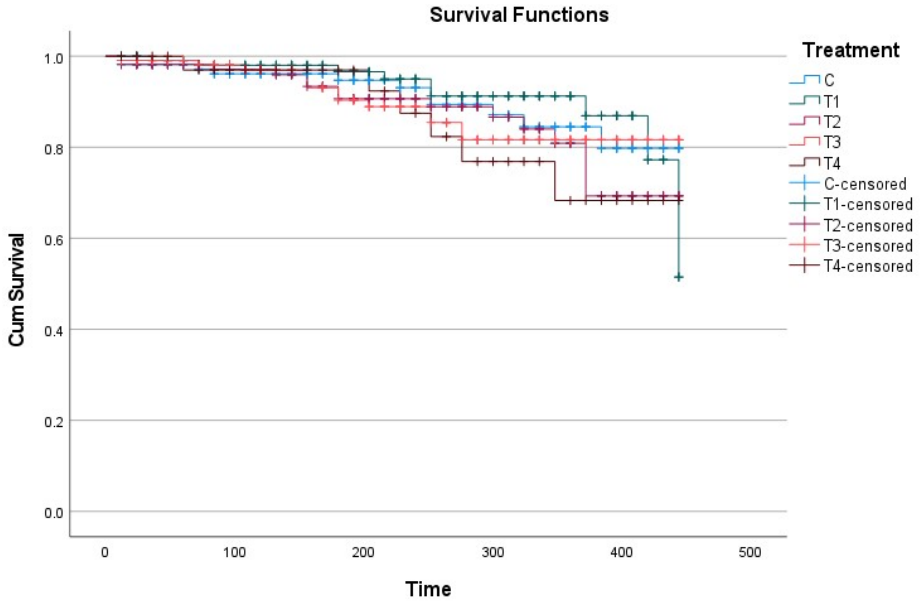

Fig. 5. Kapalan Meier survival function graph of *M. rosenbergii* over a 30-days challenge test against *Vibrio* sp. Different horizontal-coloured lines indicate censoring.

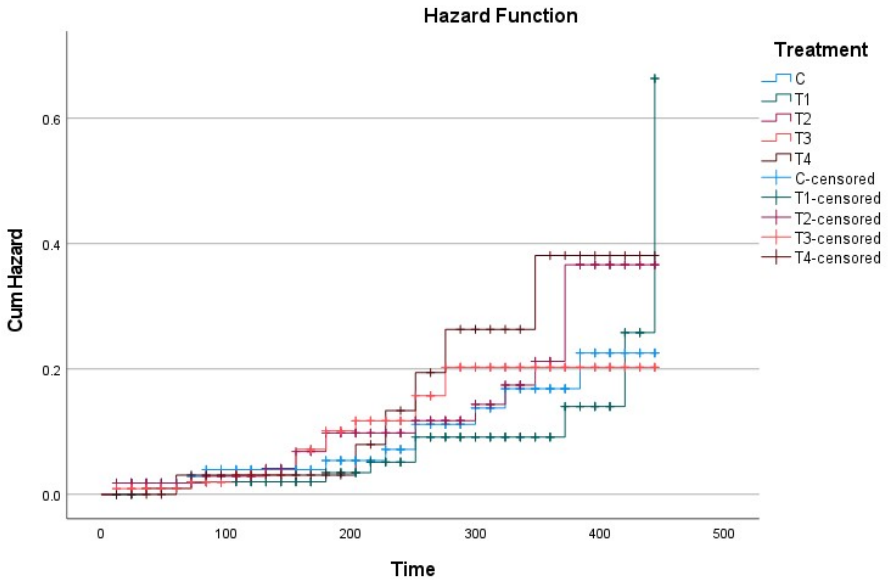

Fig. 6. Kapalan Meier hazard function graph of *M. rosenbergii* over a 30-days challenge test against *Vibrio* sp. Censoring is indicated by different horizontal-coloured lines.

Discussion

This study investigated four different concentrations of a pathogenic *Vibrio* sp. isolated from infected tiger shrimp to determine LC_{50} on *M. rosenbergii* juveniles. Regarding aquatic food-borne diseases, various virulence factors highlight *Vibrio vulnificus, V. parahaemolyticus,* and *V. cholerae* as considerably important in seafood contamination ranging from crustacean and molluscan shellfish to the giant water prawn (Rao et al. 2015).

The pathogenicity of this *Vibrio* sp. strain was determined based on probit analysis with an LC_{50} of 10^4 to 10^7 CFU/mL. Strain having LC_{50} at 10^6 CFU/mL was found more pathogenic, which was also close to 10^5 CFU/mL. Pathogenicity at these concentrations has been designated moderately pathogenic and mortality at 10^7 CFU/mL can be considered non-pathogenic (Sundell et al. 2019). Although not significant, but 50% mortality was at the lowest possible time in T3 or in other treatments might be due to the quorum-sensing effects of this bacteria, which might regulate their virulence mechanisms at certain cell densities during the challenge test period (Table I) (Jiang et al. 2019). Ruangpan and Kitao (1991) marked *Vibrio* spp. as one of the pathogenic factors, causing high mortality of farmed marine fish and shrimp in Thailand. *Vibrio cholerae*, *V. alginolyticus*, *V. carchariae* were also reported as the main pathogens from prawn hatchery in Can Tho, Vietnam (Austin 1993).

The model effects supported that different bacterial concentrations did not have any effect ($P = 0.867$) on the mortality of *M. rosenbergii*, whereas culture period might have a significant impact on prawn mortality (Tables I and II). This insignificant effect of

different concentrations might be due to the pathogenicity of this bacteria, even at lower concentrations. This phenomenon might also happen due to the significant growing capability of this strain in the culture environment which could reach quorum sensing concentrations during a fleeting period (Poopandi et al. 2021). *Vibrio harveyi* showed around 200-fold higher maximal quorum sensing-regulated bioluminescence when associated with larvae than in the culture water (Defoirdt and Sorgeloos 2012). In this experiment, no significant differences were found in the survival of *M. rosenbergii* at the end of the experiment, which further indicated that this *Vibrio* sp. can be equally pathogenic from the cell densities 10^4 CFU/mL to 10^7 CFU/mL. *V. parahaemolyticus* was found to exhibit 80% mortality in an immersion challenge test at 2×10^9 CFU/ml, which was not found pathogenic at lower concentrations (e.g., 2×10^9 and 2×10^9 CFU/ml) (Khuntia et al., 2008). *Vibrio parahaemolyticus* has several virulence factors with which it can survive on or within the aquatic hosts, especially the giant freshwater prawn, *M. Rosenbergii* (Hameed et al. 2003). Vibrios, including the species *Vibrio harveyi*, *V. alginolyticum*, *V. vulnificus*, *V. fischeri* and *V. parahaemolyticus* isolated from diseased tiger shrimp showed phospholipase, lipase, protease, and haemolytic based virulence activity which may be responsible for their pathogenesis (Manilal et al. 2010).

Within this 30-days challenge test, the cumulative survival probability of *M. rosenbergii* was higher in T1 compared to other treatments, possibly due to lower bacterial concentrations throughout the experimental period. In contrast, a lower chance of cumulative survival in T2 might be due to higher bacterial activity towards virulence or virulence regulating quorum sensing mechanism. The probability of cumulative hazard to mortality of prawn in this study was higher in T2, which is closed to T3 compared to T1. Surprisingly, it was found a bit higher in T4 might be due to other factors or molecular and physiological mechanisms. However, the probability of cumulative hazard in T1 indicates that prawns might be infected by lower concentrations of *Vibrio* sp. (Fig. 6). Similarly, *Vibrio* spp. isolated from a diseased *M. rosenbergii* showed high pathogenicity in a bath challenge test towards prawn juveniles at the cell density of 1×10^5 CFU/ml (Azad et al. 2019). *V. harveyi* was found pathogenic towards *M. rosenbergii* larvae, showed 60% mortality at day 8 of an immersion challenge test. However, quorum sensing disrupting molecules cinnamaldehyde and the thiophenone increased the survival of prawn larvae, which indicates that quorum sensing might be an important virulence regulator of vibrios (Pande et al. 2013). This study suggests that pathogens isolated either from infected *P. monodon* or *M. rosenbergii*, may be virulent to both of this commercially important species. Therefore, comprehensive research should be conducted to investigate the virulence activity of pathogenic isolates and examine their pathogenicity towards hosts within the major cultivable crustacean species.

This study shows that LC_{50} was observed at the bacterial density of 10^6 CFU/mL. The mortality of prawn juveniles was not affected by different concentrations of *Vibrio* sp., except its exposure time.Cumulative survival probability of *M. rosenbergii* was higher at 10^4 CFU/mL compared to other cell densities. So, more lower concentrations should be considered to investigate the pathogenicity of this isolate of bacterium. However, more molecular mechanisms, including virulence and virulence regulatory functions (e.g.,

quorum sensing) of this bacterium should be studied to better understand their effects on prawn culture.

Literature Cited

Amar, M. J. S. A., Caipang, C. M. A., Lopez, J. D. M., Murillo, M. N. A., Amar, E. C., Piñosa, L. A. G., & Pedroso, F. L. (2022). *Proteus mirabilis* (MJA 2. 6S) from saline tolerant tilapia exhibits potent antagonistic activity against *Vibrio* spp., enhances immunity, controls NH3 levels and improves growth and survival in juvenile giant tiger shrimp, *Penaeus monodon*. *Aquaculture Research*, *53*(16), 5510–5520.

Austin, B. (1993). Environmental issues in the control of bacterial diseases of farmed fish. *Environment and Aquaculture in Developing Countries. ICLARM Conf. Proc. 31,* 237–251.

Azad, M. A. K., Islam, S. S., Amin, M. N., Ghosh, A. K., Hasan, K. R., Bir, J., Banu, G. R., & Huq, K. A. (2021). Production and economics of probiotics treated *Macrobrachium rosenbergii* at different stocking densities. *Animal Feed Science and Technology*, *282*, 115125. https://doi.org/10.1016/j.anifeedsci.2021.115125

Azad, M. A. K., Islam, S. S., Ghosh, A. K., Hasanuzzaman, A. F. M., Smith, A. J., Bir, J., Ahmmed, M. K., Ahmmed, F., Banu, G. R., & Huq, K. A. (2023). Application of zymetin and super PS probiotics in hatchery, nursery, and growout phases of Macrobrachium rosenbergii and their impact on culture environment, production, and economics. *Journal of the World Aquaculture Society*, *54*, 645-665.

Azad, M. A. K., Islam, S. S., Sithi, I. N., Ghosh, A. K., Banu, G. R., Bir, J., & Huq, K. A. (2019). Effect of probiotics on immune competence of giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture Research*, *50*(2), 644–657.

Barua, P., & Rahman, S. H. (2020). Aquatic health index of coastal aquaculture activities at Southeastern Coast of Bangladesh. *Water Conservation Management*, *4*(2), 53–69.

Bonami, J.-R., & Widada, J. S. (2011). Viral diseases of the giant freshwater prawn *Macrobrachium rosenbergii:* a review. *Journal of Invertebrate Pathology*, *106*(1), 131–142.

Briggs, M. (2013). Analysis of freshwater prawn hatchery problems in Bangladesh. *Report to USAID Funded. Feed the Future Aquaculture (FtF AQ) Project of the World Fish Center, Bangladesh and South Asia Office*.

Bruto, M., James, A., Petton, B., Labreuche, Y., Chenivesse, S., Alunno-Bruscia, M., Polz, M. F., & le Roux, F. (2017). *Vibrio crassostreae*, a benign oyster colonizer turned into a pathogen after plasmid acquisition. *The ISME Journal*, *11*(4), 1043–1052.

de Souza Valente, C., & Wan, A. H. L. (2021). Vibrio and major commercially important vibriosis diseases in decapod crustaceans. *Journal of Invertebrate Pathology*, *181*, 107527.

Defoirdt, T., & Sorgeloos, P. (2012). Monitoring of *Vibrio harveyi* quorum sensing activity in real time during infection of brine shrimp larvae. *The ISME Journal*, *6*(12), 2314–2319.

DoF. (2018). Yearbook of Fisheries Statistics of Bangladesh, 2017-18. *Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh: Ministry of Fisheries.*, *35*, 129.

Hameed, A. S. S., Rahaman, K. H., Alagan, A., & Yoganandhan, K. (2003). Antibiotic resistance in bacteria isolated from hatchery-reared larvae and post-larvae of *Macrobrachium rosenbergii*. *Aquaculture*, *217*(1–4), 39–48.

Hodgson, E., & Rose, R. L. (2008). Metabolic interactions of agrochemicals in humans. *Pest Management Science: Formerly Pesticide Science*, *64*(6), 617–621.

Hooper, C., Debnath, P. P., Stentiford, G. D., Bateman, K. S., Salin, K. R., & Bass, D. (2023). Diseases of the giant river prawn *Macrobrachium rosenbergii*: A review for a growing industry. *Reviews in Aquaculture*, *15*, 738-758.

Islam, S. S. (2016). *Impact of quorum sensing on the virulence of Vibrio crassostreae and Vibrio tasmaniensis in vitro and in vivo in blue mussel larvae*. MS thesis, Ghent University, Belgium. 74p.

Islam, S. S., Zhang, S., Eggermont, M., Bruto, M., Le Roux, F., & Defoirdt, T. (2022). The impact of the multichannel quorum sensing systems of *Vibrio tasmaniensis* and *Vibrio crassostreae* on virulence towards blue mussel (*Mytilus edulis*) larvae. *Aquaculture*, *547*, 737414.

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.

Jewel, M. A. S., Haque, M. A., Rahman, M. H., Khatun, M. S., Akter, S., & Bhuyain, M. A. B. (2021). Shrimp polyculture: An economically viable and environmentally friendly farming system in low saline coastal region of Bangladesh. *Iranian Journal of Fisheries Sciences*, *20*(6), 1649–1663.

Jiang, Q., Chen, J., Yang, C., Yin, Y., & Yao, K. (2019). Quorum sensing: a prospective therapeutic target for bacterial diseases. *BioMed Research International*, *2019*.

Joseph, T. C., Murugadas, V., Reghunathan, D., Shaheer, P., Akhilnath, P. G., & Lalitha, K. v. (2015). Isolation and characterization of *Vibrio cholerae* O139 associated with mass mortality in *Penaeus monodon* and experimental challenge in postlarvae of three species of shrimp. *Aquaculture*, *442*, 44–47.

Khuntia, C. P., Das, B. K., Samantaray, B. R., Samal, S. K., & Mishra, B. K. (2008). Characterization and pathogenicity studies of Vibrio parahaemolyticus isolated from diseased freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Aquacult. Res., 39*(3), 301–310.

Kuçukgul Gulec, A., Altinterim, B., & Aksu, O. (2013). Determination of lethal concentration (LC50) values of *Cinnamomum zeylanicum* hydrosol on carp fish.*Iranian Journal of Fisheries Sci.,12(1),* 34-44.

Lavilla-Pitogo, C. R., Baticados, M. C. L., Cruz-Lacierda, E. R., & de la Peña, L. D. (1990). Occurrence of luminous bacterial disease of Penaeus monodon larvae in the Philippines. *Aquaculture*, *91*(1–2), 1–13.

Mamun, A.A. (2020). Influence of salinity on aquaculture species richness in the mangrove-river connected zone of southwest Bangladesh. *Aquaculture, Aquarium, Conservation & Legislation*, *13*(4), 2180–2195.

Mandal, B., Dubey, S. K., Ghosh, A. K., & Dash, G. (2015). Parasitic occurrence in the giant freshwater prawn *Macrobrachium rosenbergii* from coastal West Bengal, India. *Journal of Parasitology and Vector Biology*, *7*(6), 115–119.

Manilal, A., Sujith, S., Selvin, J., Shakir, C., Gandhimathi, R., & Kiran, G. S. (2010). Virulence of vibrios isolated from diseased black tiger shrimp, *Penaeus monodon*, Fabricius. *Journal of the World Aquaculture Society*, *41*(3), 332–343.

Mastan, S. A., & Begum, S. K. A. (2016). Vibriosis in farm reared white shrimp, *Litopenaeus vannamei* in Andhra Pradesh-natural occurrence and artificial challenge. *International Journal of Applied Sciences and Biotechnology*, *4*(2), 217–222.

Mondal, D. K., Begum, N., Rahman, S. L., & Islam, M. L. (2021). Culture potential of fresh water giant prawn (*Macrobrachium rosenbergii*) with tiger shrimp (*Penaeus monodon*) and green back mullet (*Chelon subviridis*) in poly culture.*International Journal of Fisheries and Aquatic Science*, *9*(1), 230-234.

Ninawe, A. S., & Selvin, J. (2009). Probiotics in shrimp aquaculture: avenues and challenges. *Critical Reviews in Microbiology*, *35*(1), 43–66.

Pande, G. S. J., Scheie, A. A., Benneche, T., Wille, M., Sorgeloos, P., Bossier, P., & Defoirdt, T. (2013). Quorum sensing-disrupting compounds protect larvae of the giant freshwater prawn *Macrobrachium rosenbergii* from *Vibrio harveyi* infection. *Aquaculture*, *406*, 121–124.

Phatarpekar, P. V, Kenkre, V. D., Sreepada, R. A., Desai, U. M., & Achuthankutty, C. T. (2002). Bacterial flora associated with larval rearing of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture*, *203*(3–4), 279–291.

Phuoc, L. H., Corteel, M., Nauwynck, H. J., Pensaert, M. B., Alday Sanz, V., Van Den Broeck, W., Sorgeloos, P., & Bossier, P. (2008). Increased susceptibility of white spot syndrome virus infected *Litopenaeus vannamei* to *Vibrio campbellii*. *Environ. Microb., 10*(10): 2718–2727.

Poopandi, S., Sundaraj, R., Rajmichael, R., Thangaraj, S., Dhamodharan, P., Biswal, J., Malaisamy, V., Pandian, C. J., & Jeyaraman, J. (2021). Computational screening of potential inhibitors targeting MurF of *Brugia malayi* Wolbachia through multi-scale molecular docking, molecular dynamics and MM-GBSA analysis. *Molecular and Biochemical Parasitology*, *246*, 111427.

Rao, R., Bing Zhu, Y., Alinejad, T., Tiruvayipati, S., Lin Thong, K., Wang, J., & Bhassu, S. (2015). RNA-seq analysis of *Macrobrachium rosenbergii* hepatopancreas in response to *Vibrio parahaemolyticus* infection. *Gut Pathogens*, *7*(1), 1–16.

Ruangpan, L., & Kitao, T. (1991). Vibrio bacteria isolated from black tiger shrimp, *Penaeus monodon* Fabricius. *Journal of Fish Diseases*, *14*(3), 383–388.

Siripornadulsil, W., Thongserm, M., & Siripornadulsil, S. (2014). Pathogenicity of halophilic *Vibrio harveyi* in giant freshwater prawns (*Macrobrachium rosenbergii* De Man). *Aquaculture Research*, *45*(12), 1979–1988.

Soto-Rodríguez, S. A., Simoes, N., Roque, A., & Gil, B. G. (2006). Pathogenicity and colonization of *Litopenaeus vannamei* larvae by luminescent vibrios. *Aquaculture*, *258*(1–4), 109–115.

Sundell, K., Landor, L., Nicolas, P., Jørgensen, J., Castillo, D., Middelboe, M., Dalsgaard, I., Donati, V. L., Madsen, L., & Wiklund, T. (2019). Phenotypic and genetic predictors of pathogenicity and virulence in *Flavobacterium psychrophilum*. *Front. Microb.*, *10*, 1711.

Sung, H.-H., Hwang, S.-F., & Tasi, F.-M. (2000). Responses of giant freshwater prawn (*Macrobrachium rosenbergii*) to challenge by two strains of *Aeromonas* spp. *Journal of Invertebrate Pathology*, *76*(4), 278–284.

Sung, Y. Y., Pineda, C., MacRae, T. H., Sorgeloos, P., & Bossier, P. (2008). Exposure of gnotobiotic Artemia franciscana larvae to abiotic stress promotes heat shock protein 70 synthesis and enhances resistance to pathogenic *Vibrio campbellii*. *Cell Stress and Chaperones*, *13*, 59–66.

Volkmer, B., & Heinemann, M. (2011). Condition-dependent cell volume and concentration of *Escherichia coli* to facilitate data conversion for systems biology modeling. *PloS one*, *6*(7), e23126.

Wang, L., Chen, Y., Huang, H., Huang, Z., Chen, H., & Shao, Z. (2015). Isolation and identification of *Vibrio campbellii* as a bacterial pathogen for luminous vibriosis of *Litopenaeus vannamei*. *Aquaculture Research*, *46*(2), 395–404.

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