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Mud Crab Aquaculture Challenges and Strategies for Sustainable Management

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Abstract: A study was conducted at the Fishery Harbor, Nizampatnam, Bapatla District, Andhra Pradesh, India, to evaluate bacterial populations, the physicochemical quality of water and soil, and their potential impacts on crab aquaculture and environmental health. Soil, water, and crab samples were collected for microbiological and physicochemical analyses. The study identified five bacterial species: *Pseudomonas sp., Vibrio sp., Klebsiella sp., Staphylococcus sp.,* and *Escherichia coli, with Vibrio sp.* emerging as the most prevalent, particularly in crab intestines, indicating its significant role in the gastrointestinal environment.The physicochemical assessment of soil and water provided insights into factors influencing bacterial distribution. Biochemical tests, including Gram staining, motility, and enzymatic activity assessments, offered a deeper understanding of the metabolic profiles and environmental adaptations of these bacterial species.These findings underscore the necessity for ongoing monitoring and the implementation of effective management strategies to control bacterial diseases. Such measures are crucial for enhancing the sustainability of crab aquaculture and ensuring the health of the Fishery Harbor ecosystem. The results emphasize the importance of targeted interventions to mitigate bacterial impacts on environmental health and aquaculture productivity, thereby supporting the long-term viability of the region's crab farming industry.

Keywords: Bacterial Analysis, Fishery Harbor, Nizampatnam, Crab Farming, Microbiological Study.

1.0 Introduction

Mud crabs (Scylla spp.) are highly esteemed seafood commodities traded extensively in India and other regions (Fielder and Allen, 2004; Kathirvel et al., 2004). The aquaculture of mud crabs has been practiced for decades across various

Asian countries, including India, where it plays a pivotal role in the seafood industry (Kathirvel et al., 2004). Despite its potential, mud crab aquaculture faces significant challenges, particularly in disease management. Diseases such as White Spot Syndrome Virus (WSSV), bacterial infections, parasitic infestations, shell diseases, and gill infections pose severe threats to mud crab populations. These diseases can cause high mortality rates, reduced yields, and increased production costs, thereby impacting the economic sustainability of the industry.In India, mud crab farming primarily involves fattening soft-shelled or water crabs, employing straightforward methods aimed at enhancing marketability (Kathirvel, 1993; Marichamy, 1996; Marichamy and Rajapackiam, 2001; Patterson and Samuel, 2005; Sakthivel and Francis, 2006).

Biosecurity Protocols: Implementing stringent measures is crucial to prevent the introduction and spread of diseases within crab farms. This includes controlling facility access, disinfecting equipment, and monitoring the health status of incoming stocks (FAO, 2011).

Water Quality Management: Maintaining optimal water quality parameters such as temperature, dissolved oxygen levels, salinity, and pH is critical for the health and growth of mud crabs. Poor water quality can stress crabs, making them more susceptible to diseases (Hargreaves, 2006).

Health Monitoring: Regular monitoring of crab health through visual inspections and diagnostic tests aids in early disease detection. Prompt identification allows for timely intervention and reduces the risk of disease outbreaks (Bondad-Reantaso et al., 2005).

Disease Treatment and Management: Developing effective treatment protocols for common diseases like WSSV and bacterial infections is crucial. This may involve the use of antimicrobial agents, probiotics, or immunostimulants, depending on specific pathogens and local regulatory guidelines (Lightner, 2011).
Research and Innovation: Continued research into disease prevention, genetics, nutrition, and farming techniques is crucial for the sustainable growth of mud crab aquaculture. Innovations in breeding for disease resistance and improving feed formulations can enhance productivity and profitability (Bhairamadgi et al., 2017).

1.1 Bacterial Necrosis:
Bacterial necrosis, commonly observed in larvae, post-larvae, or adults, presents as 'black spot', 'brown spot', 'burnt spot', 'shell disease', or chitinolytic bacterial disease. It is caused by chitinolytic bacteria that degrade the chitin in the exoskeleton, resulting in erosion and melanization (dark brown to black pigmentation) at the

infection site. Implicated Gram-negative rods include Vibrio spp., Pseudomonas spp., Aeromonas spp., and Spirillum spp. (Lavilla-Pitogo et al., 2001).

1.2 Filamentous Bacterial Diseases:

Filamentous bacterial diseases caused by organisms such as *Leucothrixmucor, Thiothrix spp.,* and *Flexibacter spp.* can lead to mortality through gill discoloration and subsequent secondary infections.

1.3 Luminescent Bacterial Disease:

Luminescent bacterial disease, severe and economically significant, primarily results from Vibrio species and related genera (ShanmugaPriya, 2008). Vibriosis affects marine and estuarine shellfish species and often occurs secondary to poor water quality, stress, and inadequate nutrition. Symptoms in affected adults include reduced appetite, stunted growth, darkened hepatopancreas, and mass mortalities. *V. harveyi* particularly infects crab larvae rearedin hatchery conditions, displaying fluorescence under dark light and reducing feeding, sometimes resulting in extensive mortalities.Infections involving multiple bacterial species, viruses, fungi, or parasites are common. Co-infections with V. harveyi and V. campbellii have been reported in mud crabs infected with WSSV (Poornima et al., 2008), and other Vibrio species such as *V. vulnificus, V. nereis, V. fischeri,* and *V. fluvialis* have also been identified (ShanmugaPriya, 2008).

2.0 Materials and Methods

2.1 Study Area

The research was carried out at the Fishery Harbor in Nizampatnam, Bapatla District, and Andhra Pradesh, India (522314). The harbor, spanning an area of approximately 21,782 square meters, currently operates with a fleet of around 500 boats (Government of Andhra Pradesh, 2020)

2.2 Sample Collection

Samples of soil, water, and crab were collected from various locations within the Fishery Harbor to ensure representative coverage. Crab samples were sourced from vendors at the harbor's landing center during early morning hours (between 7:00 and 8:00 local time) as per Kumar et al. (2018).

2.3Collection of Water and Soil Samples

Water and soil samples were gathered in October 2023 from two distinct locations, with multiple replicates at each site. Soil samples were stored in zip-lock poly bags, while water samples were collected in sterile Falcon tubes.

2.4 **Soil Analysis:**Soil organic matter (SOM) was determined by adding potassium dichromate (1N) and sulfuric acid to a known soil sample, followed by titration with ferrous ammonium sulfate.The percentage of calcium carbonate was measured by reacting soil with hydrochloric acid and titrating the resulting solution with sodium hydroxide.

2.4 Water Analysis: Soil water content was calculated by drying soil samples at 110 ± 5°C and measuring the weight difference.Water quality was assessed based on several physicochemical parameters:pH: Measured using the electrometric method according to Indian Standard IS 3025: Part 12.Other Parameters: Color, odor, turbidity, temperature, specific conductance, total dissolved solids, and major cations and anions were evaluated to understand water quality dynamics (APHA, 2017).

2.5Microbiological Analysis

All glassware was washed, dried, and sterilized. Culture media were autoclaved at 121°C for 15 minutes (Adibe and Eze, 2004).

Sample Preparation: For bacteriological analysis, approximately 10 g of crab sample was homogenized and mixed with 90 mL of peptone water (Obi and Krakowiaka, 1983).

Serial Dilution: Serial dilutions were prepared by transferring 1 mL of the sample into 9 mL of sterile peptone water, repeating the process to achieve dilutions ranging from 10^-1 to 10^-6.

Bacterial Isolation: Soil, water, and crab samples were plated on nutrient agar and incubated at 37°C for 24-48 hours to facilitate bacterial growth. The glass spreader technique was used to inoculate duplicate nutrient agar plates with diluted samples.

Counting of Bacterial Load: Colony counts were determined using the method outlined by Collins et al. (1989). Countable plates with 1 to 32 colonies were selected, and bacterial loads were expressed in colony-forming units per milliliter (CFU/mL).

2.7 Oxidase Test: Determined the presence of oxidase enzyme by color change on filter paper.

2.8 Catalase Test: Tested for catalase production by observing bubbling after adding hydrogen peroxide.

2.9 Starch Hydrolysis: Identified bacteria capable of hydrolyzing starch by the clear zone formation around colonies after iodine staining.

2.10 Gelatin Hydrolysis: Tested gelatinase production by observing gelatin liquefaction.

2.11 Indole Test: Determined indole production from tryptophan using Kovács reagent.

2.12 Methyl Red Test: Assessed mixed acid fermentation using methyl red indicator.

2.13 Voges-Proskauer Test: Evaluated acetoin production using specific reagents.

3.0 Results

3.1 Biochemical Characterization of Bacterial Species

The following table summarizes the results of biochemical tests used to differentiate between five bacterial species: Pseudomonas sp., Vibrio sp., Klebsiella sp., Staphylococcus sp., and Escherichia coli.

3.1 Gram Staining: Pseudomonas sp., Vibrio sp., Klebsiella sp., and E. coli are Gram-

negative, indicating a thinner peptidoglycan layer and the presence of an outer membrane.Staphylococcus sp. is Gram-positive, characterized by a thicker peptidoglycan layer in its cell wall.

3.1 Motility: Pseudomonas sp., Vibrio sp., and E. coli are motile, suggesting the presence of flagella which facilitate movement.Klebsiella sp. and Staphylococcus sp. are non-motile, indicating the absence of flagella or other motility structures.

3.1 Oxidase Test: Vibrio sp. is oxidase-positive, reflecting the presence of cytochrome c oxidase, an enzyme involved in the electron transport chain.The other species are oxidase-negative, indicating a lack of this enzyme.

3.1 Catalase Test: All tested species are catalase-positive, which means they produce the enzyme catalase that decomposes hydrogen peroxide into water and oxygen.

3.1 Starch Hydrolysis: Only Staphylococcus sp. shows positive starch hydrolysis, demonstrating the production of amylase that breaks down starch into simpler sugars.

3.1 Gelatin Hydrolysis: Pseudomonas sp., Vibrio sp., and E. coli are gelatinase-positive, indicating they can hydrolyze gelatin to its constituent amino acids.Klebsiella sp. is gelatinase-negative.

3.1 Indole Test: Pseudomonas sp., Vibrio sp., and E. coli are indole-positive, indicating they produce indole from tryptophan via the enzyme tryptophanase.Klebsiella sp. and Staphylococcus sp. are indole-negative.

3.1 Methyl Red Test: Pseudomonas sp., E. coli, and Staphylococcus sp. are methyl red-positive, indicating a mixed acid fermentation pathway with a low pH.Vibrio sp. and Klebsiella sp. are methyl red-negative.

3.1 Voges-Proskauer Test: Vibrio sp., Klebsiella sp., and Staphylococcus sp. are Voges-Proskauer positive, indicating the production of acetoin as a fermentation end product.Pseudomonas sp. and E. coli are Voges-Proskauer negative.

3.1 **Citrate Utilization Test:**Vibrio sp., Klebsiella sp., and Staphylococcus sp. are citrate-positive, meaning they can utilize citrate as the sole carbon source.Pseudomonas sp. and E. coli are citrate-negative.

3.2 **Urease Test:**Klebsiella sp., Staphylococcus sp. are urease-positive, showing the ability to hydrolyze urea into ammonia and carbon dioxide.Pseudomonas sp., Vibrio sp., and E. coli are urease-negative.

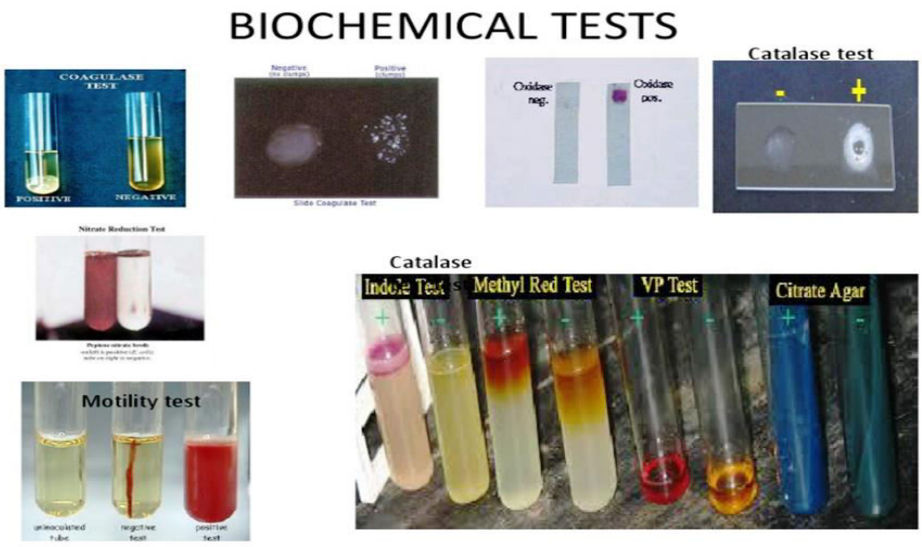
BIOCHEMICAL TESTS
COAGULASE TEST
POSITIVE
NEGATIVE
Oxidase neg.
Oxidase pos.
Catalase test
Nitrate Reduction Test
Motility test
Catalase
Indole Test
Methyl Red Test
VP Test
Citrate Agar

Table 1: Distribution of Bacterial Colonies in Various Samples

S.No	Microorganisms	% of Colonies in Fish Samples	Soil	Water	Crab Head	Crab Intestine	Total
1	*Pseudomonas sp.*	18 %	7	4	4	3	18
2	*Vibrio sp.*	34 %	6	7	7	14	34
3	*Klebsiella sp.*	13 %	8	1	2	2	13
4	*Staphylococcus sp.*	9 %	5	2	1	1	9
5	*E. coli*	11 %	6	1	2	2	11

3.2 Distribution of Bacterial Colonies in Various Samples

***1. Vibrio sp.*:**Vibrio sp. is the most abundant bacterial species identified, with a total of 34% of colonies found across all samples.Notably, Vibrio sp. shows the highest count in the crab intestine (14), which suggests a significant presence in the digestive environment of the crab.This high prevalence in the crab intestine could indicate an adaptation to the gastrointestinal conditions or a higher availability of suitable nutrients.

2. Pseudomonas sp:Pseudomonas sp. contributes to 18% of the total colonies. It is relatively less abundant in the crab intestine, with only 3 colonies identified.Its presence in the soil, water, and crab head is higher, suggesting a broader environmental distribution.

3. Klebsiella sp.:Klebsiella sp. accounts for 13% of the total colonies. It is less prevalent in the crab intestine (2 colonies) compared to other environments such as soil.This may indicate that Klebsiella sp. is less adapted to the crab intestine environment but is more common in soil.

4. E. coli:

E. coli represents 11% of the colonies. Similar to Klebsiella sp., it has a low presence in the crab intestine (2 colonies).Its higher presence in soil and water suggests it

might be more commonly associated with these environments rather than the crab intestine.

5.Staphylococcus sp:

Staphylococcus sp. has the lowest prevalence, with only 9% of total colonies. Its count in the crab intestine is minimal (1 colony), indicating it is not a dominant species in the crab's gut environment.The higher numbers in soil suggest that *Staphylococcus sp.* might be more commonly found in environmental settings rather than the crab intestine.

3.4 Biochemical Test Insights

The biochemical test results(as shown in Fig 4) can provide further insights into the metabolic capabilities and identification characteristics of these bacterial species. For instance:

Motility: Vibrio sp., Pseudomonas sp, and E. coli are motile, which might aid their survival and colonization in diverse environments, including the crab intestine.

Oxidase and Catalase Tests: Variations in these tests help distinguish between the species and understand their metabolic profiles, which can be linked to their prevalence in different environments.

4.0 Conclusion

The data highlights *Vibrio sp*. as the most prevalent species in the crab intestine, with significant representation in other environments as well. In contrast, *Staphylococcus sp*. is the least prevalent overall. The biochemical characteristics of these species provide additional context for their distribution and prevalence in different environments, underscoring their ecological roles and adaptations.This detailed analysis provides a comprehensive understanding of the bacterial colonization patterns and the environmental adaptations of different bacterial Species in crab sample. In conclusion, while mud crab aquaculture presents significant economic opportunities, effective disease management is paramount for its sustainable development. By implementing robust biosecurity measures, maintaining high water quality standards, and adopting proactive health monitoring and treatment strategies, stakeholders can mitigate disease risks and ensure the long-term viability of the industry. Conclusion: Crab farming in India is a rapidly expanding industry, but it faces significant challenges due to bacterial diseases, particularly shell rot caused by Vibrio spp. This infection compromises the crabs'

resilience, leading to increased mortality rates, reduced yield, and decreased profitability. Effective disease management strategies are crucial to address this issue. Regular bacterial examinations of crab populations and their culturing environments, such as the Fishery Harbor in Nizampatnam, Bapatla district, are vital for early detection and intervention. Implementing strict biosecurity measures and adopting best practices in aquaculture can minimize the spread of bacterial diseases, ensuring the sustainable growth of the crab cultivation industry in India. Through comprehensive biochemical analysis, five distinct bacterial species were identified from the collected samples: Pseudomonas, Vibrio, Klebsiella, Staphylococcus, and Escherichia coli (E. coli). These findings highlight the importance of monitoring and controlling bacterial populations in crab farming environments to prevent disease outbreaks and promote a healthy and productive industry.

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Table 02 Biochemical test

Biochemical Test	***Pseudomonas sp.***	***Vibrio sp.***	***Klebsiella sp.***	***Staphylococcus sp.***	***E. coli***
Gram Staining	Negative	Negative	Negative	Positive	Negative
Motility	Motile	Motile	Non-motile	Non-motile	Motile
Oxidase Test	Negative	Positive	Negative	Negative	Negative
Catalase Test	Positive	Positive	Positive	Positive	Positive
Starch Hydrolysis	Negative	Negative	Negative	Positive	Negative
Gelatin Hydrolysis	Positive	Positive	Negative	Positive	Positive
Indole Test	Positive	Positive	Negative	Negative	Positive
Methyl Red Test	Positive	Negative	Negative	Positive	Positive
Voges-Proskauer Test	Negative	Positive	Positive	Positive	Negative
Citrate Utilization Test	Negative	Positive	Positive	Positive	Negative

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