Challenges and Solutions in Shrimp Aquaculture: Advances in Microbial Identification

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Introduction

Shrimp aquaculture in India has long been considered a high-risk, high-reward industry. However, in recent years, this notion has lost its relevance as farmers face mounting challenges in both production and sales. Rising raw material prices, coupled with declining exports due to intense international competition, high production costs, anti-dumping duties, export taxes, stringent regulations, and the absence of a robust local market and supply chain infrastructure, have created a crisis for the shrimp aquaculture market in India.

On the production front, farmers are battling persistent growth and disease issues. The repeated use of the same soil and water, unpredictable weather fluctuations driven by climate change, the shrimp's primitive immune system, and the mismatch between brooder genetics and the evolving environment are key factors contributing to the recurring failure of shrimp cultures in India. When these failures occur, farmers often attribute the problems to either brooder genetics or poor seed quality from hatcheries.

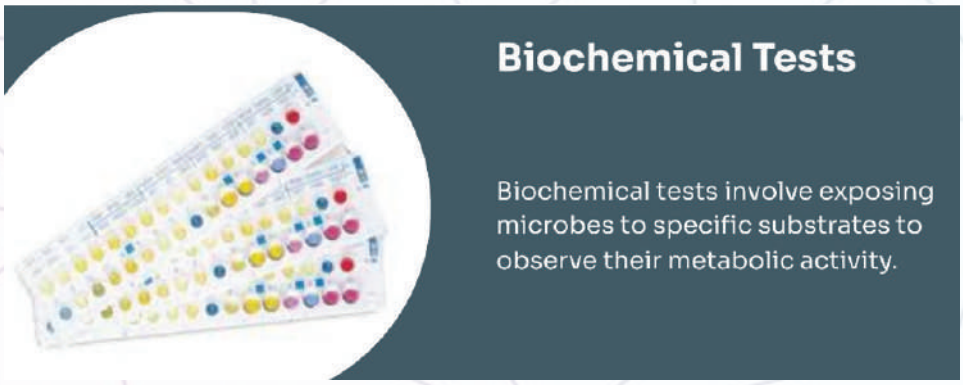
Hatcheries, the starting point of Indian aquaculture, manage the entire cycle from brooder importation to rearing. However, they face the same disease challenges as farmers. Despite adhering to strict Standard Operating Procedures (SOPs) and maintaining advanced infrastructure, hatcheries are struggling as pathogens become increasingly resilient, particularly in a fluctuating environment. Compounding the issue, shrimp lack a mature immune system, making it essential to identify pathogens and adapt SOPs for sustainable hatchery production. For the past decade, Zoea 2 syndrome has been a significant challenge in hatcheries, but more recently, the M3-PL problem has emerged with a similar impact, causing substantial survival losses or stalled conversion at the M3-PL stage, much like Zoea 2 syndrome. Both forms of pathogenicity have led to significant financial losses for hatcheries. Identifying pathogens is, therefore, the critical first step toward understanding their impact and revising SOPs—the only path forward for sustainable hatchery production. This approach also applies to farming.

Microbial Identification: Evolving Techniques

Historically, identifying microbes was a complex and time-consuming task, requiring numerous tests, staining procedures, a well-equipped laboratory, and skilled technical personnel. The process often took days of experimentation to reach a definitive conclusion. Thanks to recent technological advancements, microbial identification has become more efficient. Today, biochemical tests and staining can be conducted using strip methods or automated systems, significantly reducing the time and effort required. In this article, we explore several microbial identification systems and understand the knowledge on antibiotic resistance/sensitivity that can be effectively utilized in aquaculture to address the growing challenge of disease management.

Conventional Biochemical Tests

Conventional biochemical tests involve labour-intensive procedures, including multiple staining techniques and slow-paced experiments. These methods require various chemicals and a proper laboratory set-up to ensure accurate execution. Typically, these procedures can take between 24 to 48 hours to complete.



The primary advantage of these tests is that they do not require costly equipment. In recent years, semi-automated strips, such as the API (Analytical Profile Index) system, have become available in the market. While these strips allow for quicker experiments, they do not support an electronic database for storing results, and their cost can be relatively high. Consequently, many laboratories now prefer fully automated systems for faster, electronically recorded results over conventional biochemical tests.

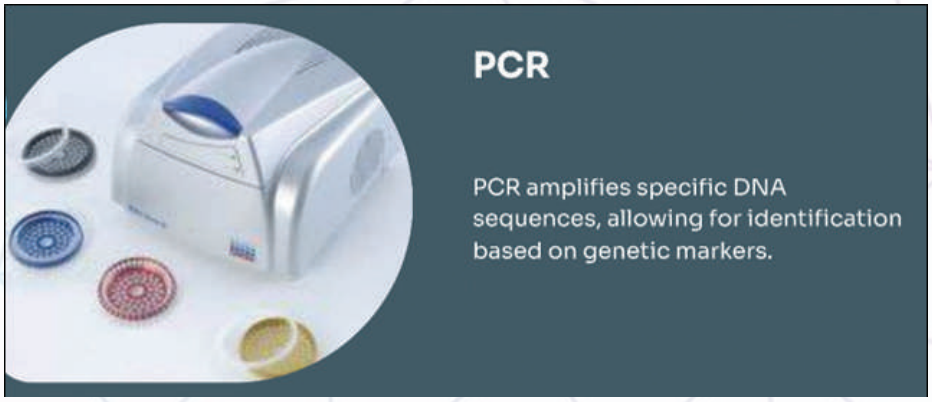
VITEK 2 Compact (BioMerieux)

In many hospitals and clinical laboratories, fully automated phenotypic identification systems like the VITEK 2 Compact (BioMerieux), BD Phoenix System (Becton Dickinson), and MicroScan System (Thermo Fisher Scientific) are widely used. These automated machines can perform up to 64 biochemical tests simultaneously, enabling rapid microbial identification in a short time. In aquaculture, the adoption of such automated systems allows for quick pathogen identification, facilitating prompt precautionary measures to mitigate the damage caused by pathogens. For instance, a study identified pathogens such as Vibrio alginolyticus and *V. harveyi* in market shrimp samples using conventional biochemical tests, VITEK 2, and MALDI-TOF MS (Sanhoury, et al., 2016). Similarly, a survey of Chinese snails in a seafood market using VITEK 2 identified all pathogenic isolates as *V. parahaemolyticus* (Song, et al., 2020). In practice, shrimp pathogens such as *Vibrio spp., Aeromonas, Photobacterium, and Streptococcus* can be isolated using specific media and subjected to VITEK 2 for rapid identification (Sanhoury, et al., 2016).

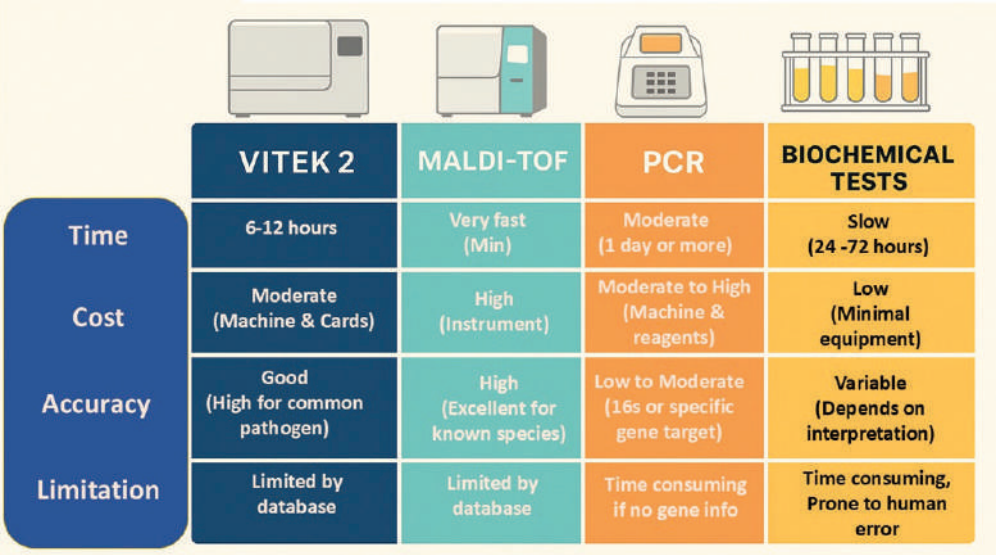
The VITEK 2 system includes a comprehensive biochemical dataset for common bacteria, allowing for easy identification. However, it has limitations, as it may struggle to identify rare or new species. Misidentifications have also been reported in some cases. For example, *V. cholerae* was misidentified as another species (Saini, et al., 2012), and *Aeromonas veronii* clinical strains were misidentified as *V. alginolyticus* (Park, et al., 2003). Therefore, unusual shrimp isolates or unexpected biochemical profiles should be rechecked using more advanced methods like PCR, MALDI-TOF MS, or sequencing. Additionally, the VITEK 2 system's database must be regularly updated with new or rare bacterial biochemical profiles to improve its microbial identification capabilities.

PCR Identification

Polymerase Chain Reaction (PCR) is a molecular-based technique that offers significant advantages for gene- or species-specific identification of known



bacterial targets. If the target gene for a particular bacterial species is known, PCR enables rapid identification—within a few hours. This method is particularly effective for identifying closely related species with high accuracy, provided prior information about the target conserved gene is available. For example, genes such as dnaJ (species-specific) and toxR (virulence gene) are commonly used to identify Vibrio alginolyticus in shrimp isolates (Sanhoury, et al., 2016). Without prior gene information, identification often relies on 16S rRNA or other marker gene assays combined with sequencing, which can be time-consuming. However, when prior gene information is available, species-specific marker genes can be designed, allowing for easy detection of the pathogen either from an isolated culture or directly from the sample.



	VITEK 2	MALDI-TOF	PCR	BIOCHEMICAL TESTS
Time	6-12 hours	Very fast (Min)	Moderate (1 day or more)	Slow (24 -72 hours)
Cost	Moderate (Machine & Cards)	High (Instrument)	Moderate to High (Machine & reagents)	Low (Minimal equipment)
Accuracy	Good (High for common pathogen)	High (Excellent for known species)	Low to Moderate (16s or specific gene target)	Variable (Depends on interpretation)
Limitation	Limited by database	Limited by database	Time consuming if no gene info	Time consuming, Prone to human error

MALDI-TOF MS

Matrix-Assisted Laser Desorption Ionization–Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is another powerful technique for bacterial identification, relying on protein fingerprint sequences. Similar to the VITEK 2 system, MALDI-TOF MS requires a pure bacterial culture for sample processing. This method offers faster identification and higher accuracy compared to VITEK 2 (Guo, et al., 2014). When a pure culture is available, MALDI-TOF MS can identify bacteria in just a few minutes, with a high accuracy rate if the system's database is comprehensive. Additionally, the per-sample cost for testing is lower compared to other methods. However, the main drawback is the high cost of the instrument itself, which is significantly more expensive than other systems. The species-level error rate for broad clinical samples using MALDI-TOF MS is approximately 5.6%, compared to 6.2% for VITEK 2 (Guo, et al., 2014). MALDI-TOF MS excels at distinguishing Vibrio species accurately; for instance, one study reported 100% accuracy in detecting V. cholerae and 99% accuracy for V. parahaemolyticus (Banerjee, et al., 2025).

Comparison of Microbial Identification Systems

To provide a clearer understanding of the strengths and limitations of the microbial identification systems discussed, the following chart compares Conventional Biochemical Tests, VITEK 2 Compact, PCR, and MALDI-TOF MS based on key factors such as speed, accuracy, cost, and limitations.

A Case Study: Applying Advanced Techniques in Aquaculture

We, Amazing Biotech Pvt. Ltd., technical team focuses on assaying bacteria for their probiotic potential and bioremediation capabilities. We operate three service-based laboratories dedicated to supporting shrimp culture for the benefit of farmers. In both hatcheries and farms, diseases remain a major challenge, often leading to significant economic losses.

Therefore, the rapid identification of pathogenic microbes is critical for determining the causative agent and developing effective remedies or SOPs to ensure sustainable aquaculture.

Traditionally, our labs relied on conventional biochemical assays to identify both probiotic and pathogenic strains. Recently, we partnered with Dr. Seghal Kiran from Pondicherry University and began using the advanced VITEK 2 Compact system for faster identification. This system has enabled us to obtain rapid results, allowing us to respond to issues promptly. By identifying the pathogen, we can develop targeted probiotic solutions or establish improved SOPs, benefiting both hatcheries and farms.

Often, when a problem arises in a farm or hatchery, the immediate response is to harvest or drain the tank without understanding the root cause. A new culture may then be started without revising the SOP, leaving the issue unaddressed. If the pathogen persists or the same flawed protocol is followed, the problem is likely to recur, potentially leading to further economic losses. Identifying the causative agent is, therefore, essential to prevent such setbacks.

Recently, several hatcheries along the Chennai-Pondicherry and Ulavupadu coasts have faced significant M3-PL problems. We collected samples from some of these hatcheries and processed them using the VITEK 2 Compact system and PCR methods. For further analysis, we sent the samples for sequencing. Through these techniques, we successfully identified the causative agent and recommended improved SOPs for the hatcheries. Moreover, once we obtained prior gene information about the causative agent, a simple PCR test allowed us to detect and quantify the pathogen, enabling early implementation of precautionary protocols. This approach significantly reduced economic losses. The same methods can be applied to farms as well.

Conclusion

Incorporating advanced automated techniques in aquaculture is crucial for farmers to reduce disease prevalence. Adopting improved Standard Operating Procedures (SOPs) provides a strategic advantage in combating proliferating virulent pathogens and addressing climate change challenges. In this article, we explore various microbial identification systems and their applications. A case study demonstrates how VITEK 2 and PCR techniques helped revise SOPs in hatcheries. Increased adoption of these techniques can empower farmers to overcome diseases and achieve sustainable aquaculture.

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