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Advancing Fish Health Management: The Role of Next Generation Sequencing (NGS)

Tanuja Abdulla*, Thankam Theresa Paul and Deepa Sudheesan

ICAR-Central Inland Fisheries Research Institute, Kochi Research Station, Kochi, Kerala (682 018), India

Corresponding Author

Tanuja Abdulla

✉: tanujacifri@gmail.com

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Abstract

Next Generation sequencing techniques were applied in the field of fish and aquatic health management studies over the last few years. The era of genomic sequencing advances from Sanger sequencing to Illumina sequencing, 454 Pyrosequencing, SOLiD Sequencing, Oxford nanopore sequencing etc. with advantage of sequencing of millions of DNA reads in short time with reasonable cost. This tool helps in the detailing of microbial information associated with fish and environmental parameters like fish diseases, host-pathogen interaction studies, pathogen characterization and microbial community diversity assessments. NGS techniques enables novel identification of un-cultivable microbes, bio-prospecting of probiotic and bioremediation bacteria and identification of different strains of pathogens those are difficult to differentiate with conventional diagnostic methods. This information is vital for the development of management strategies in the aquatic ecosystem. This article briefly highlights the application of NGS tools in fish health aspects.

Keywords: Fish health, Microbiome, Next generation sequencing, Pathogenesis

Introduction

Next-Generation Sequencing (NGS) is emerging as a powerful molecular biology tool involves high-throughput, fast and big data sequencing of millions of DNA molecules. Compared to the single gene detection, the data retrieval volume of NGS ranges to hundreds of millions short reads and long reads of DNA in a single sequence run. These cost-effective sequencing platforms are generated in the early 2000s and revolutionized the genomics and transcriptomics understanding of biological processes such as breeding traits, disease resistance strains, host-pathogen interaction studies and microbial profiling. Compared to the known and single gene assays, the NGS offers information regarding the known and unknown origin of millions of genomic data. High throughput data analysis with the use of advanced sequencing enables the researchers to understand genomic information in short time period. The development of next generation sequencing helps more precise documentation of the microbial community structure and composition compared to isolation and identification of separate samples.

NGS Approach in Fish Health

Fish health management practices intended to prevent the occurrence of diseases and thereby enhancing fish production by adopting measures such as disease-free stocks, periodic health monitoring, specific diagnosis and therapeutic measures. Metagenomics, Metatranscriptomics, Whole-genome sequencing and RNA sequencing are the few NGS techniques used in these aspects.

Application of NGS in Fish Health

NGS tools can be used in the fish health management in various aspects (Figure 1) and the applications of NGS are briefly explained as follows.

1. Disease Diagnosis and Therapeutics

NGS facilitates the understanding of precise diagnosis of known and novel pathogens that are often not identified by traditional diagnostic methods. Illumina sequencing of genomic DNA of *Flavobacterium columnarae* Pf1 (Zhang *et al*., 2016) identifies bacterial pathogenic factors involved in fish pathogenesis. Whole genome profiling of fish and

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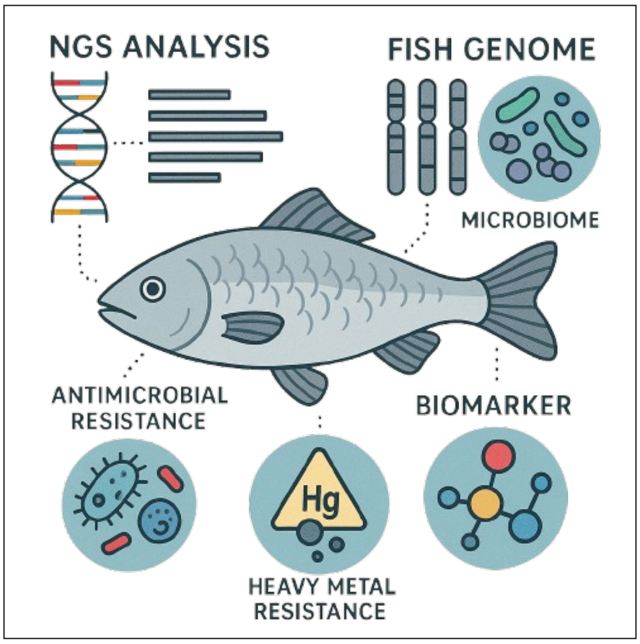

Figure 1: Schematic representation of NGS in fish health

shell fish bacterial pathogens like *Aeromonas hydrophila*, *Aeromonas veronii*, *Edwardsiella tarda*, *Vibrio vulnificus* and *Vibrio* sp. Strain B183 enabled the detailing of virulence genes and outer membrane proteins and their functional relationship with fish diseases. Ion torrent and Illumina sequencing revealed ten segmented genomes of Tilapia Lake Virus (Bacharach *et al*., 2016) in *Tilapia niloticus*. Whole genomic understanding of fish and shell fish viral pathogens enable segment wise characterization and novel genotype detection of viral strains in Viral Haemorrhagic Septicaemia Virus, *Silurus glanis* papillomavirus, Carp Edema Virus, Infectious Spleen and Kidney Necrosis Virus, White spot syndrome virus, Decapod Hepanhamaparvovirus, Decapod hepandensovirus 1 and Taura syndrome virus. Pathogenic characterization is vital for the drug development against these pathogens. Transcriptome sequence data of microbes helps to identify biologically significant genes and their functional relationship with fish diseases, will be useful in recognize the reliable therapeutic targets for fish diseases. Similarly, whole genome sequencing aids to disclose and differentiate the segment wise characterization of viral genome to identify and target reliable therapeutic target for vaccine development.

2. Biomarker Profiling

Inland aquatic ecosystem is vulnerable to pollution from industrial and anthropogenic sources and these changes are often associated with onset of fish mortality cases. The development of biomarkers serves as early warning signals for the disease preparedness. The expression changes in certain genes can be used as biomarkers specific to stress, pollution and diseases. Transcriptomic study of Rita fish (Mitra *et al.*, 2020) revealed that genes like cox7a2 (Cytochrome c oxidase polypeptide 7A2), MURC (muscle related coiled-coil protein) and Hsp70-14 (Heat Shock Protein 70 subunit 14) were regarded as pollution biomarkers in the aquatic system.

3. Microbial Resistance Gene Studies

Antibiotic resistance and heavy metal resistance in microbial population arises due to the indiscriminate use of antibiotics and heavy metal pollution in the aquatic environment. Metagenomics and Metatranscriptomics technologies enable the understanding of this resistance from the fish microbiome. Fish farms with multiple uses of antibiotics reported with antibiotic resistant bacterial strains and antibiotic-resistant genes (ARG's) (Mohammed *et al*., 2025) through whole genomic profiling of resistant bacteria. Heavy metals such as copper, zinc, cadmium and lead resistance genes regarded as Heavy metal resistance genes (HMRGs) namely copA, czcA, ncc, merA and ars (resistance to copper, cadmium, nickel, mercury and arsenic) are found in bacteria that colonize in fish gut and other tissues. Suitable drug alternatives, antimicrobial awareness and efficient effluent management system are essential to mitigate these microbial resistances in the aquatic ecosystem. Antimicrobial resistance surveillance programs are the need of hour to monitor, report and identify hotspots to regulate the use of antibiotics and chemicals in the aquatic system.

4. Microbiome Profiling

Shot gun metagenomics aids the functional profiling of microbial communities present in the fish associated with various habitats, diseases, immunity and metabolism. Microbiome studies on the fish gut and mucus unveils the potential of this technology to understand the host-microbe interactions, novel screening of microbes, microbiome diversity in ecological niches and bio-prospecting for new therapeutic agents. Microbiome abundance analysis through 16S rRNA Illumina sequencing of gut microbiome of the *Carassius auratus* and skin microbiome of Atlantic salmon (de Bruijn *et al.*, 2018) revealed difference in the bacterial community composition in the diseased and healthy fishes.

Conclusion

The significance of genome studies envisages "*One Day One Genome*" initiative for cataloguing bacterial species present in environment, agriculture and human health by Department of Biotechnology (DBT) and Biotechnology Research and Innovation Council (BRIC) of India in the year 2024. The microbial profiling of fish and shell fish pathogens will be helpful to document and develop new strategies and control measures for fish health management in fisheries and aquaculture.

Future Prospects

The emergence of new pathogens necessitates the requirement of fast and advanced technical interventions to understand the pathogenic mechanism and to develop suitable drugs against different pathogenic strains for the management measures to prevent production losses in the aquaculture system.

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