OPINION

Next-generation sequencing technologies and the improvement of aquaculture sustainability of Pacific white shrimp (*Litopenaeus vannamei*)

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At a global level, the Pacific white shrimp (*Litopenaeus vannamei*) is the main cultivable crustacean species. It currently accounts for a generous proportion of the total production of shrimp, as well as a large proportion of the animal protein supply to society. However, the presence of viruses and bacteria, the use of brood stocks based on phenotypic selections that do not guarantee the genetic traits of interest in shrimp development, and feeding with diets other than fishmeal have impacted its growth, nutrition and increased its susceptibility to infectious diseases resulting in great mortality, which has reduced the total production.

Considering the importance and economic value of *L. vannamei* in aquaculture, it is necessary to develop and apply novel tools that support the search for solutions to the main problems influencing its production. One alternative is the omic technologies based on next-generation sequencing (NGS). Details of NGS, as well as its application in different areas of scientific research during the last ten years, have been extensively reviewed by Goodwin *et al.*[1]; they will not be discussed here. However, a general summary will be provided regarding contributions on the development and application of the NGS technique in research on the evolution, growth, nutrition, reproduction and resistance to infectious diseases to improve the sustainability of *L. vannamei* aquaculture.

To support the sustainability of the Pacific white shrimp culture, we must understand its genetic and genomic characteristics with reference to the complete genome of *L. vannamei*. However, assembling the genome has been difficult due to a high percentage (~80) of repetitive genomic sequences, although work continues to obtain a white shrimp reference genome. Recently, an NGS technique called restriction site-associated DNA sequencing (RADseq) has been used to develop a high-quality genetic map that has generated molecular markers related to shrimp growth. From the data obtained, it has been possible to estimate a genomic size of 2.6 GB (ref. 2). Therefore, it is essential to continue research for a genomic reference of *L. vannamei* by performing hybrid assemblies with data obtained from different NGS platforms. This allows complete genome sequencing, and consolidation of structural and functional genomics needed to understand the mechanisms of evolution, adaptation, gene regulation and resistance to pathogens, which will accelerate the genetic improvement of *L. vannamei*.

NGS technology has enabled the development and application of a transcriptomic analysis of the Pacific white shrimp through massive RNA sequencing (RNAseq). The transcriptome of pathogen-free *L. vannamei* larvae has revealed the possible functions of the genes and proteins involved in the route of infection caused by *Vibrio parahaemolyticus*, indicating that the shrimp has evolved its immune response mechanisms against this bacterial infection[3]. In another study, the transcriptome of a male pathogen-free shrimp collected in Sonora, Mexico was analysed, and the genes involved in DNA replication as well as the binding proteins and catalytic activity were reported, while the antioxidant response molecules were not significantly represented[4]. These data have greatly enriched the available genetic and genomic information about *L. vannamei.* It can thus be used as a reference to determine the differences in the gene expression in shrimp larvae and juveniles at growing stages when they are vulnerable to infection by viruses or bacteria. This is because transcriptomic data have only been obtained in shrimp challenged with viral pathogens under experimental conditions, but never in farmed shrimp. For example, experiments with *L. vannamei* exposed to Taura syndrome virus (TSV) and white spot syndrome virus (WSSV) have found significant differences in the genetic expression of the prophenoloxidase activation system, phagocytosis and cellular apoptosis, which play important roles in the immune response. In addition, differences have been found in the mitogen-activated protein kinase processes, which contribute to viral replication[5–8]. Transcriptomics has broadened our understanding of the interactions between *L. vannamei* and viral pathogens at the molecular level. Future research may lead to the discovery of molecular therapies that could silence the metabolic pathways which accelerate viral replication or enhance the metabolic pathways of the immune response to address health and mortality problems in *L. vannamei* cultures.

The RNAseq technique has also been used to analyse the gonadal transcriptome in *L. vannamei* reproductive organs. Genes that are involved in important metabolic pathways of the reproductive processes of shrimp have also been reported in oocytes and gonadal maturation, sexual differentiation, follicular development, and those related to the use of fat diets[9,10]. These data can be used for marker-assisted selection to promote the use of high-quality broodstocks that guarantee the inheritance of genetic traits of interest that enhance maturation, growth, nutrition and tolerance to stress and pathogens in the development of shrimp larvae for a sustainable *L. vannamei* aquaculture.

Transcriptome analysis using NGS has also been used to analyse the influence of diets on gene expression by developing nutrigenomics in *L. vannamei*. This study was conducted by Mexican researchers who evaluated the influence on gene expression of a diet based on *Ulva clathrata*. They concluded that the incorporation of plant proteins influences lipid metabolism, immune response, stress tolerance and oxidation–reduction processes at the molecular level[11]. Nevertheless, much research is still needed to address shrimp nutrition problems. Thus, we must continue a nutri-genomic approach to investigate how alternative

diets to traditional fish raw materials (meal and oil) influence gene expressions. This will help identify families of *L. vannamei* that adapt better and present a favourable growth response to generate molecular markers for the genetic selection of breeding shrimp that produce *L. vannamei* larvae with high nutritional yields. For the nutrition problem, it would also be interesting to develop the metagenomics for the complete genomic sequencing of microbiomes of the stomach and intestine of shrimp adapted to diets other than fishmeal and fish oil. This study would provide a better insight into how microorganisms influence digestion and the immune response mechanisms, and will help identify and isolate microorganisms that function as probiotics promoting the adaptation of shrimp to experimental diets with a beneficial impact on nutrition, growth and disease resistance.

In summary, the development and application of omic technologies (genomics, transcriptomics, nutrigenomics and metagenomics) based on NGS and bioinformatic support may be the key to solving the problems of the global aquaculture of *L. vannamei*, thereby improving the industry's sustainability profile.

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