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Chapter 7

Current Advances in Functional Genomics in Aquaculture

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Abstract

Gene expression studies in aquaculture have slowly evolved from the traditional reductionist approach of single gene sequencing to high throughput sequencing (HTS) techniques able to sequence entire genomes of living organisms. The upcoming of HTS techniques has led to emergence of metagenomics, nutrigenomics, epigenetics and other omics technologies in aquaculture in the last decade. Metagenomics analyses have accelerated the speed at which emerging pathogens are being discovered, thereby contributing to the design of timely disease control strategies in aquaculture. Using metagenomics, it is easy to identify and monitor microbial communities found in different ecosystems. In vaccine production, genomic studies are being used to identify cross neutralizing antigens against variant strains of the same pathogens. In genetics and epigenetics, genomics traits have been identified that are beginning to gain commercial applications in aquaculture. Nutrigenomics have not only enhanced our understanding of the biological markers for nutrition-related diseases, but they have also enhanced our ability to formulate diets able to maintain a stable immune homeostasis in the gut. Overall, herein, we have shown that functional genomics provide multifaceted applications ranging from monitoring microbial communities in aquatic environments to optimizing production systems in aquaculture.

Keywords: genomics, aquaculture, metagenomics, nutrigenomics, epigenetics

1. Introduction

The ability to decipher the molecular composition of nucleic acids of living organisms is of prime importance in biological sciences. Although the traditional approaches of single gene expression analyses using polymerase chain reaction (PCR) tests [1, 2], quantitative real

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time PCRs (qRT-PCRs) [3, 4], competitive PCRs [5] or nested PCRs [6] have been and are still widely used in biological sciences, they inherently lack the ability to provide a global overview of genomic transcripts found in living organisms. However, the recent advent of omics technologies such as metagenomics, nutrigenomics and epigenetics based on high throughput sequencing (HTS) is rapidly enhancing our ability to understand complex systems underlying different biological functions. These omics technologies have not only accelerated whole genome sequencing projects of different aquatic organisms [7, 8], but they also have the capacity to unravel the sequences of entire genomes without prior knowledge of the genes to be sequenced thereby enhancing the discovery and annotation of novel genes in non-model species. And as shown from recent studies, their applications in aquaculture have accelerated our ability to identify emerging pathogens [9], monitor the microbiomes of different aquatic environments [10], develop nutritional diets with less side effects [11, 12] and understand the cellular networks that regulate different biological processes in aquatic organisms [13–15]. It is evident from studies carried out this far that an integrated use of different omics technologies is bound to improve our production systems in aquaculture [10, 12, 16–18]. Hence, this chapter provides an overview of different omics technologies currently used in aquaculture mainly focusing on their overall contribution to transforming genomics studies into functional applications.

2. Application of metagenomics analyses

Studies carried out this far show that metagenomics can be used to identify novel pathogens as well as microbiota found on mucosal surfaces of cultured aquatic organisms.

2.1. Application of metagenomics in diagnostics and discovery of novel pathogens

The rapid expansion of aquaculture to become a leading source of proteins for human consumption in the world has brought with it a rapid increase in the number pathogens infecting farmed aquatic organisms [19]. To expedite the process of identifying emerging pathogens, there has been a shift in recent years from the use of traditional diagnostic tools based on isolation, culture and pathogen characterization to include metagenomics analyses in the identification of novel pathogens in aquaculture [10]. Metagenomics is a culture independent diagnostic tool that does not require prior knowledge of nucleic acids to be sequenced unlike conventional PCR that require prior knowledge of the nucleic acid to be sequenced for the design of primers [20]. Metagenomics analyses have the capacity to sequence all nucleic acids present in a sample at once thereby generating a vast array of data that requires computational analyses for interpretation [20, 21]. As pointed out in our previous studies [9, 10], it has the advantage of identifying co-infections and in the case of viral pathogens, it has the capacity to generate all variable proteins that form complete virions thereby permitting comparative phylogenetic analyses with other viruses present in public databases. Moreover, it is a proactive diagnostic tool able to identify novel pathogens before they cause outbreaks unlike the reactive traditional diagnostic tools in which etiological agents are only identified after

causing disease outbreaks reaching epidemic proportions [21]. Using metagenomics, several novel pathogens have been identified at a much faster rate than traditional approaches in which the duration from first observation of clinical signs to identification of the pathogens is long [10]. For example, infectious pancreatic necrosis (IPN) was first reported as an acute infectious enteritis [22] in salmonids in the 1940s while the etiological was later characterized as IPN virus after 20 years in 1960 [23]. Similarly, viral haemorrhagic septicaemia (VHS) was first reported in the early 1950s in salmonids while the causative agent was characterized later after 10 years in 1962 [24]. This trend was observed for several other diseases such as infectious hematopoietic necrosis virus (IHNV), nervous necrosis virus (NNV), heart and skeletal muscle inflammation (HSMI) and cardiac myopathy syndrome (CMS) in which identification of the etiological agents took long after clinical signs were first reported [25–33]. However, the upcoming of metagenomics has accelerated our discovery of novel pathogens in which the duration from observation of first clinical signs to identification of the etiological agent has been reduced. In fish, viruses discovered using metagenomics include circoviruses from common bream [34] and European eel [35], posavirus [36] and seadornavirus [37] from freshwater carp and totivirus from golden shiner. As shown in our recent study [9], more than 20 novel fish pathogenic viruses have been identified using metagenomics in the last 4 years, which is more than the number identified using traditional diagnostic tools in the last 5 decades, clearly showing the rapid rate at which metagenomics has accelerated our ability to identify novel pathogens compared with traditional diagnostic methods.

In crustaceans, mortalities due to white spot syndrome virus (WSSV) in shrimps were first reported in 1992 while the causative agent was identified in 2001 [38–40]. Mortalities due to taura syndrome virus (TSV) in shrimps were first reported in Ecuador in 1991 [41] and the virus was characterized in 1994 [42]. A similar trend was observed for Yellow heard disease virus (YTV) [43, 44], infectious hypodermal and hematopoietic necrosis virus (IHHNV) [45–47], shrimp infectious myonecrosis virus (SIMV) [48] and *Penaues vannamei* nodavirus (PvNV) [49, 50] in which the duration between the first report of the disease and identification of the etiological agent was long. Shrimps viruses discovered using metagenomics analyses include *Frafantepenaeus duorarum* nodavirus (FdNV) and shrimp hepatopancreas-associated circular nodavirus (ShrimpCDV) [51].

2.2. Monitoring of environmental microbiomes

A good understanding of microbial communities found in freshwater and marine environment used for aquaculture is a prerequisite to designing effective disease control strategies tailored for each ecosystem. Metagenomics analyses provide a unique opportunity to study infectious agents in water samples outside their susceptible hosts [10]. Its ability to sequence all nucleic acids present in a sample at once enables it to profile microbial communities found in different ecosystems. For example, Angly et al. [52] showed that microbial composition varies with latitude gradient with highest diversity being in warm climates around the equator and less diversity in the poles. After analysis of viromes from 32 different marine sites, Dinsdale et al. [53] noted that viral richness decreased from deep sea to surface waters and with distance from shore in surface waters and increased from winter to summer. Given that

over 40% of the global human population live within 100 km of coastlines, anthropogenic activities have been shown to influence the composition of microbial communities in coastal areas where aquaculture activities are mostly carried out [54]. These anthropogenic activities include host species composition changes introduced by aquaculture [55, 56], waste disposal [57], agriculture [58], recreation [59] and industrial activities [59]. As a result, metagenomics is currently being used to monitor the impact of anthropogenic activities on coastal microbial composition. Port et al. [60] found an increase in antibiotic resistance genes caused by coastal effluent discharges, while Morán et al. [61] showed significant changes in bacterial community structures caused by coastal copper disposal in La Lancha and Chañaral bay in the Pacific Ocean. Overall, these studies show that metagenomics is not only used to identify novel pathogens, but it is also used to monitor the impact of human activities on microbial composition in different aquatic environments.

2.3. Application of metagenomics in recirculation systems

In contrast to outdoor aquaculture systems that are dependent on natural water basins such as rivers and oceans, the recirculation aquaculture system (RAS) uses water that is filtered before it is recycled back into culture tanks in closed systems. Water used in RAS is subjected to several treatment processes such as biofiltration to reduce ammonium, removal of solids, oxygenation, pH control and pathogen denaturation using ozone and UV-light. Although a well-designed state-of-the-art RAS has the potential to reduce the presence of waterborne microorganisms, some pathogens are able to resist RAS disinfection. Bacteria phyla detected from RAS biofilters include Actinobacteria [62], Firmicutes, Bacteroides [63–65], Protobacteria [63, 65], Verrucomicrobia [65] and Sphingobacteria [62, 65]. Hence, some microorganisms are being used as biosafety indicators whose dominance points to increase in the proliferation of pathogenic microorganisms [66]. As a result, metagenomics analyses are being used to monitor the increase in proliferation of pathogens in RAS [67].

2.4. Metagenomics analyses of mucosa microbiota

Given that mucosal surfaces are the major portals of microbial invasion, there has been a growing interest to study mucosal microbiota of cultured aquatic organisms. Metagenomics studies show that different environmental factors influence the composition of mucosal microbiota on different fish species.

2.4.1. Skin mucosa microbiota

Larsen et al. [68] compared the skin microbiota of six different fish species (*Mugil cephalus*, *Lutjanus campechanus*, *Cynoscion nebulosus*, *Cynoscion arenarius*, *Micropogonias undulatus* and *Lagodon rhomboides*) from the Gulf of Mexico and showed that Proteobacteria was the predominant phylum followed by Firmicutes and Actinobacteria across all species. Although Aeribacillus was found in 19% of all fish species examined, genera such as Neorickettsia and Microbacterium were fish species-specific pointing to existence of phyla and genera associated

with particular fish species. Lokesh and Kiron [69] showed that the bacterial operational taxonomy unit (OTU) composition on the skin of Atlantic salmon (*Salmo salar* L.) changed significantly as a result of transfer from fresh to seawater. Proteobacteria was the dominant phylum both in fresh and seawater while Bacteroidetes, Actinobacteria, Firmicutes, Cyanobacteria and Verrucomicrobia were the most abundant in freshwater. The genus *Oleispira* was the most abundant in seawater. Similarly, Wilson et al. [70] showed that bacterial communities from the epidermal mucus of Atlantic cod (*Gadus morhua*) from the Baltic, Iceland and North seas collected over three seasons mainly comprised of Psychrobacter, Bacteroides and Photobacterium OTUs in all seasons although there were significant inter-site and seasonal variations in community composition.

Boutin et al. [71] combined 16S RNA metagenomics and QTL analyses to show that host genotype can regulate the microbiota composition on the skin surface of brook charr (*Salvelinus fontinalis*). They found a strong negative correlation between Flavobacterium and Methylobacterium, pointing to a mutually competitive relationship between pathogenic and non-pathogenic bacteria on the skin mucosa of brook charr. Flavobacterium is known to be pathogenic among different fish species, while Methylobacteria provide protection against pathogenic bacterial infections on skin surfaces suggesting that a shift from Methylobacteria to Flavobacterium dominance on the skin mucosal could point to increase in susceptibility to bacterial infection. Hence, by monitoring changes on mucosal bacteria composition, metagenomics can be used to determine the susceptibility of fish to microbial infections.

2.4.2. Gut mucosal microbiota

As pointed out by Lyons et al. [72] that to better understand the gut microbiome and its impact on the health status of aquatic organisms, it is vital to determine its structure, diversity and potential functional capacity. Gajardo et al. [12] analysed the microbiota profile of the digesta and gut mucosal of Atlantic salmon (*S. salar* L.) fed commercial diets and showed that microbiota richness and diversity differed significantly between the digesta and gut. The digesta had a higher and diverse richness than the gut mucosa. Proteobacteria was the dominant phyla in the mucosa whereas Proteobacteria and Firmicules were dominant in the digesta. In addition, there were significant differences in microbiota composition in different segments of the gut. Actinobacteria was dominant in the posterior intestinal (PI) than the mid-intestinal (MI) mucosa. Moreover, the PI showed presence of Spirochaetes that were not found in the MI showing that metagenomics can be used to identify microbial communities that inhabit different segments of the gut. In another study, Gajardo et al. [11] identified bacterial groups associated with diet-induced gut dysfunction that could serve as biological markers of the gut health status in Atlantic salmon. Mouchet et al. [73] compared the gut microbiota of 15 fish species from the Atlantic Ocean near Brazil and showed that the microbiota genetic diversity was highly influenced by the fish species, geographical location and diet. Put together, these studies show that metagenomics can be used to profile bacteria species on mucosal surfaces of different fish species and that different factors such as host species, geographical areas and diet influence mucosal microbiota in fish.

2.5. Metagenomics technologies and their limitations

Of the most widely used NGS technologies, both 454 pyrosequencing Roche and Illumina sequencers have been widely used in the metagenomics analyses of different aquatic organisms. For example, 454 pyrosequencing Roche has been used to study microbial communities of different fish species including rainbow trout (*Oncorhynchus mykiss*) [74], Atlantic cod (*G. morhua*) [75], Atlantic salmon [76], brook trout (*S. fontinalis*) [77], brown trout (*Salmo trutta*) [78], zebrafish (*Dario rerio*) [79], Gizzard shad (*Dorosoma cepedianum*) [80], silver carp (*Hypophthalmichthys molitrix*) [81], common carp (*Cyprinus carpio*) [82], grass carp (*Ctenopharyngodon idellus*) [83], orange spotted grouper (*Epinephelus coioides*) [84] and Senegalese sole (*Solea senegalensis*) [85]. On the other hand, Illumina sequencers have been used for the analyses of microbiota found in seabass (*Lates calcarifer*) [86], blunt snout bream (*Megalobrama amblycephala*) [87], grass carp (GC) [87], mandarin fish (*Siniperca chuatsi*) [87], topmouth culter (*Culter alburnus*), common carp [87] and Crucian carp (*Carassius auratus*) [87], silver carp [87] and bighead carp (*Hypophthalmichthys nobilis*) [87]. In terms of assembly, both whole genome shotgun and marker gene guided sequencing have been used on different aquatic organisms. The commonly used marker gene in metagenomics analyses is the 16S ribosomal RNA (16S rRNA), which has been widely used to characterize the microbiota of different aquatic organisms including rainbow trout [88, 89], Atlantic salmon [11, 12], turbot (*Scophthalmus maximus*) [90], lamprey (*Lampetra morii*) [91] and Baleen whale [92]. Whole genome shotgun sequencing has also been widely used in the study of environmental microbial communities and pathogens infecting different aquatic organisms. The major advantage with this approach is that it can be used to sequence whole genomes of known or unknown organisms using *de novo* assemblies unlike guided marker assemblies that are dependent on a reference gene [93–96].

Despite its positive contribution to the discovery of novel pathogens and environmental monitoring of microbial communities, metagenomics has significant limitations that require the support of other tools [95]. The immense metagenome data generated using NGS technologies require the support of other tools for clustering, classification and annotation of individual sequences [95]. For *de novo* assembled sequences, the most reliable annotation approach is by homology search using reference sequences available in public databases. However, the number of existing public databases for aquatic organisms is limited, which makes it difficult to identify novel pathogens [97]. In general, functional annotation lags behind the rate at which metagenome data is generated. Alternative methods used to identify novel pathogens include motif or pattern-based identification [98, 99], phylogenetic profiling [100] and neighbourhood tree alignments [101, 102].

3. Nutrigenomics in aquaculture

Nutrigenomics is the study of the role of nutrition on gene expression. Galduch-Giner et al. [103] showed that there was specialization in the functional properties of different components of the intestinal tract of the European seabass (*Dicentarchus labrax*). They observed that

molecular markers linked to nutrient digestion and absorption were high in the anterior (AI) and middle intestine (MI) while the posterior intestine (PI) predominantly expressed genes linked to immune defence mechanisms. These observations are in line with other scientists who showed that the AI and MI are mainly responsible for nutrient digestion and absorption [104, 105] while the PI is responsible for induction of innate immune responses linked to activation of adaptive immunity in teleosts fish [106–109].

Different scientists have studied the genomic changes induced by various nutrients in the guts of different fish species. Krol et al. [110] compared the differential response of the Atlantic salmon gut to soybean meal (SBM) and fish meal (FM) as positive and negative controls for enteritis, respectively. They noted that SBM altered the gut histology and induced extensive transcriptomic changes linked to underlying mechanisms of SBM-induced enteropathy. They found 18 enriched pathways linked to inflammation and immune responses induced by SBM enteropathy. Among these were the NF-kB and IL-8 signalling pathways known to induce the synthesis of various pro-inflammatory cytokines. Phagocytic pathways such as the Fcγ receptor mediated phagocytosis and monocyte pathways were highly enriched. In another study, Torrecillas et al. [111] showed downregulation of TCRβ, COX-2, TNFα, IL-8, IL-6, IL-10, TGFβ and IgM when MHC-II was upregulated in European seabass fed to Soya-bean oil (SBO). Expression of these genes corresponded with reduced lengths of intestinal folds and mucus density in the gut. Conversely, mannan oligosaccharides (MOS) diets increased the length of intestinal folds and mucus density and upregulated MHC-CD4, COX-2, TNFα and IgM expression. Combined MOS and SBO diets reduced the harmful effects of SBO diets by moderating the downregulation of GALT-related genes. Therefore, these observations show the importance of optimizing feed formulation in order to produce balanced diets able to preserve the GALT-immune homeostasis.

Apart from soyabean, nutrigenomics have also been used to evaluate the impact of other nutrients in fish diets. Azeredo et al. [112] showed that the immune status of the European seabass was impaired by arginine dietary supplements. They observed that different cell-mediated immune markers were downregulated in fish fed 1–2% arginine diets. Leukocytes obtained from fish fed arginine diets showed low respiratory burst compared to control fish. After challenge with *Vibrio aguillarum*, fish fed arginine diet supplements showed higher mortality than control fish. Interestingly, reducing arginine levels to 0.5% in the diet supplements significantly increased respiratory burst to levels comparable with control fish. In another study, Estensoro et al. [113] showed that butyrate (BP-70 ®NOREL) helped to restore the intestinal status of marine gilthead sea bream (*Sparus aurata*) fed extremely low diets of fish meal (FM) and fish oil (FO). They observed that extremely low FO and FM diet levels significantly altered the transcriptomic profiles linked to nutrient absorption in the AI and increased expression of inflammatory, antioxidant, permeability and mucus production genes that coincided with increased granulocyte and lymphocyte presence in the PI submucosa. Interestingly, expression of these genes was restored to control values by adding butyrate (BP-70) to the feed. As pointed out by Krol et al. [110], gut transcriptomic profiling is a useful tool for testing the adverse impacts of different feeds and that understanding gut-diet interactions is a prerequisite to designing diets able to prevent induction of diet-related diseases in the gut.

Omics technologies commonly used for nutrigenomics analyses in aquaculture mainly comprise of microarray and RNA-seq. RNA-seq has been widely used to study the impact of different diets in various fish species including Atlantic salmon [114], rainbow trout [115], channel catfish (*Iactalurus punctatus*) [116], blue catfish (*Ictalurus furcatus*) [117] and zebrafish [118]. On the other hand, microarray has also been widely used to study nutrigenomics in different fish species that include Atlantic salmon, rainbow trout, Atlantic cod (*G. morhua*) and Gilthead sea bream (*S. aurata*). However, the use of RNA-seq and microarray leads to several challenges that include the need for large data processing softwares as well as the need of bioinformatics tools required for differential gene expression, network pathway, alternative splicing and gene duplication analyses. To cope with these challenges, different bioinformatics tools have been developed and new innovations are being invented to cover different aspects of quality assessment of mapped genes, mapping for *de novo* assembled genes, expression quantification, differential expression analyses, alternative splicing and network pathway analyses [119–122]. Different reviews have been published providing in-depth comparative analyses of existing tools highlighting their strengths and weakness that could serve as a guide for end users to select the most appropriate tool suitable for nutrigenomics studies in different aquatic organisms [119, 123, 124].

4. Functional genomics in vaccine development

Given that most pathogens exist as multiple strains having different antigenic proteins, the challenge in vaccine design has been to find cross protective antigens against variant strains of the same pathogen. In the case of viruses, different approaches have been used aiming at finding the most neutralizing epitopes using methods such as epitope mapping, peptide-scan and reverse genetics [125–128]. However, the upcoming of next generation sequencing (NGS) supported with current advances of bioinformatics tools is expected to expedite our ability to identify the most immunogenic proteins for vaccine production against viral diseases. For example, Ou-yang et al. [129] used bioinformatics to identify the antigenic proteins for Singapore grouper iridovirus. They used the 162 open reading frames (ORFs) of SGIV for sequence similarity searches to identify motifs, cellular locations and other prediction domains to identify the most immunogenic epitopes required for vaccine production. They identified 13 genes that were cloned to produce DNA vaccines of which three vaccines produced relative percent survival (RPS) ranging from 58.3 to 66.7% in vaccinated grouper.

In the case of bacterial vaccines, identification of protective antigens can be a challenge given that they contain several antigenic proteins such as capsular antigens, fimbriae, pili and outer membrane proteins [130–132]. Some of these proteins lead to serotype, biovar or strain differences leading to antigenic diversity within bacterial species. Hence, the challenge is to identify broad neutralizing antigens able to confer cross protection against variant bacterial strains can be a difficult task. To overcome this problem, Handfield et al. [133] developed an *in vivo* induced antigen technology (IVIAT) that uses antibodies generated from individuals infected by the bacterial strain homologous to the vaccine strain to probe for immunogenic proteins using an *in vitro* expression system. To do this, a genomic library is generated using DNA fragments from the bacteria strain to be used for vaccine production. The DNA fragments are digested using

restriction enzymes and cloned into plasmid vectors. Induced colonies of the expression library are probed using pooled sera from bacterial infected individuals as shown in **Figure 1**. Reactive clones are purified and used as vaccine candidates [133]. This technology has been widely used to identify antigenic proteins for different bacteria species such as *Streptococcus iniae* [134], *Vibrio anguillarum* [135], *Aeromonas salmonicida* [136, 137], *Edwardsiella tarda* [138] and *Streptococcus parauberis* [139]. Jia et al. [138] used the IVIAT to identify a 510 aa peptidase protein, which they used to produce a subunit vaccine against *E. tarda* in Japanese flounder. Sun et al. [134] used the IVIAT technique to identify a secretory antigen, which they designated as Sia10, and cloned it to produce a DNA vaccine against *S. iniae*. In vaccinated turbot, the Sia10 protein was detected in the muscle, liver, kidney and spleen by 7 days post-vaccination (dpv) lasting until 49 dpv. Post-challenge RPS showed 73.9 and 92.3% in fish challenged with high- and low-challenge dose, respectively. In addition, the Sia10 protein produced protective antibodies in passively vaccinated fish. In another study, Sun et al. [140] used the IVIAT method to identify a surface

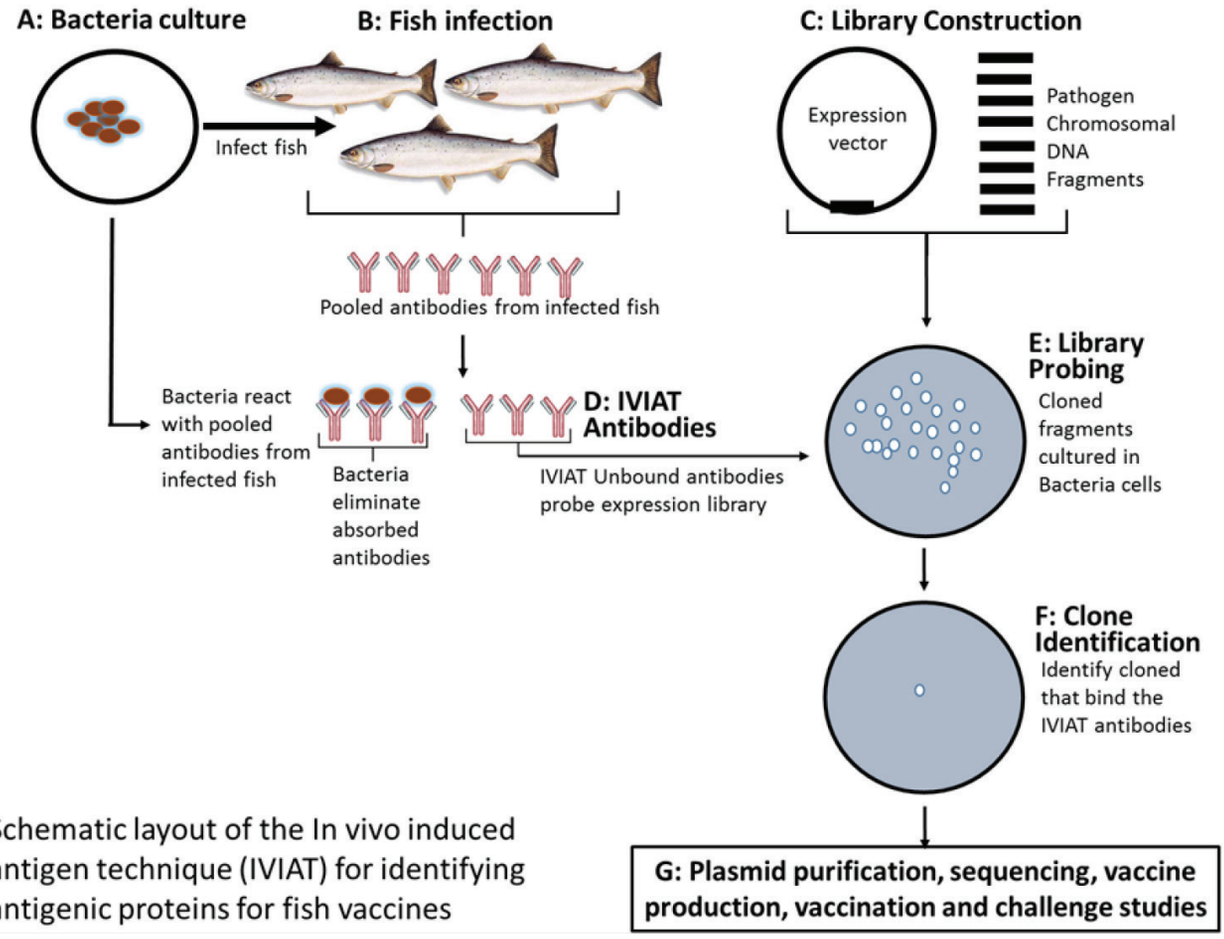


Figure 1. Schematic layout of the IVIAT technique for the identification of bacterial antigenic proteins essential for the production of fish vaccines: A: bacteria culture. B: bacteria infection in fish and the sera from infected fish is pooled. C: library construction using chromosomal DNA fragments of the bacteria cultured in (A). D: bacteria eliminate absorbed antibodies from sera while IVIAT unbound antibodies are used to probe the library constructed in (C). E: clones from fragments of bacterial chromosomal DNA are probed with IVIAT pooled sera. F: after probing with pooled sera from infected fish, clones depicting binding capacity to IVIAT sera are sub-cultured. G: the identified clones are purified, sequenced and used for subunit or DNA vaccine production followed by vaccination and challenge trials.

antigen designated as Esa1, which they used to produce a DNA vaccine against *E. tarda* in Japanese flounder. They showed that the pCEsa1 vaccine enhanced respiratory burst, acid phosphatase activity and bactericidal activity of headkidney macrophages. In addition, it produced RPS = 57% in passively vaccinated fish. Overall, these studies show that genomics approaches can be used to identify the most immunogenic proteins for different bacterial strains in order to produce the most protective vaccines for use in aquaculture.

5. Marker-assisted selection of growth and disease resistance traits

5.1. Growth traits

Genetic selection in which individuals with the best growth traits are selected as parent stock for the next generation is one of the major strategies used for improving production in aquaculture. And as such, several breeding programmes have been going on using natural selection approaches [141–143]. The major drawback with this approach is that it takes several generation cycles to identify individuals having positive growth traits. To expedite the process of identifying genetic traits for optimal growth performance, marker-assisted selection (MAS) processes such as single nucleotides polymorphism (SNP), microsatellite, amplified fragment length polymorphism (AFLP), random amplified polymorphism DNA (RAPD), restriction fragment length polymorphism (RFLP) and quantitative trait loci (QTL) are being used to scan chromosomal DNA of different farmed aquatic organisms. Among these, the most widely used is QTL analysis, which has been applied across most of the commercial fish and crustacean species used in aquaculture [144–147]. As defined by Geldermann [148], QTLs are chromosomal regions made of single genes or gene clusters determining a quantitative character of a given trait. Given their high heritability, mapped QTLs have proved to be a useful tool in selective breeding, which has played an important role in accelerating genetic improvement in aquaculture.

As shown in **Tables 1** and **2**, the most important genetic traits sought for in aquaculture are growth rate, body weight and length. These traits influence the commercial value of farmed aquatic organisms. Traits for body weight and length have been identified in several fish species such as Atlantic salmon [149], rainbow trout [150], Big heard carp (*H. nobilis*) [151], common carp [152, 153] and tilapia (*Oreochromis niloticus*) [154], nine spined stickleback (*Pungitius pungitius*) [155] and Arctic char (*Salvelinus alpinus*) [156]. In shrimps and prawns, body weight and length traits have been identified in kruma shrimp [157, 158], Chinese shrimp [159], Giant fresh water prawn [160], Ridge white prawn [161] and Oriental river prawns [162]. Another important trait, which has contributed to improved production in aquaculture is sexual maturation. It has been shown that in some some species, sex is closely related to growth. For example, Sun and Liang [163] showed that in common carp, females grow bigger than males at the same age, while in tilapia, the males grow faster than females [164]. Hence, the selection of males for aquaculture increases production in tilapia while the females increase production in carp. Important traits related to improving meat quality include muscle quality [154], muscle fibre [165], texture [165], colour [166, 167], fat percentage [166] and dressed weight percentage [166].

Fish species	Trait	Method	References
Blue bream (*Ballerus ballerus*) (Cyprinidae	Thyroid hormones	Transcriptome	[241]
Blunt snout bream (*Megalobrama amblycephala*)	Growth trait	Transcriptome	[242]
Turbot (*Scophthalmus maximus*)	Growth trait	Transcriptome	[243]
Grouper hybrids (*Epinephelus fuscogutatus*)	Superiority in growth	Transcriptome	[244]
Mandarin fish (*Siniperca chuatsi*)	Growth traits	Microsatellite	[245]
Atlantic salmon (*Salmo salar* L.)	Growth traits	SNP/GWAS	[149]
Rainbow trout (*Oncorhynchus mykiss*)	Robustness	Transcriptome	[173]
Nile tilapia (*Oreochromis niloticus*)	Growth traits	Transcriptome	[154]
Nile tilapia (*Oreochromis niloticus*)	Skeletal muscle quality	Transcriptome	[154]
gilthead sea bream (*Sparus aurata*)	Skeletal muscle quality	Transcriptome	[246]
Rainbow trout (*Oncorhynchus mykiss*)	Growth traits	SNP	[150]
Rainbow trout (*Oncorhynchus mykiss*)	Stress factor traits	Transcriptome	[247]
Atlantic cod (*Gadus morhua*)	Growth/reproduction	Transcriptome	[248]
Lake whitefish pairs (*Coregonus* spp. *Salmonidae*)	Reproduction	Transcriptome	[249]
Lake whitefish pairs (*Coregonus* spp. *Salmonidae*)	Adaptation	QTL	[250]
Atlantic salmon (*Salmo salar* L.)	Smoltification	Transcriptome	[177]
Common carp (*Cyprinus carpio*)	Cold tolerance	QTL	[163]
Arctic char (*Salnelinus alpinus*)	Temperature tolerance	QTL	[176]
Arctic char (*Salnelinus alpinus*)	Growth rate	SNP	[251]
Tilapia (*Oreochromis niloticus*)	Cold tolerance	QTL	[175]
Tilapia (*Oreochromis niloticus*)	Fish size	QTL	[175]
Coho salmon (*Oncorhynchus kisutch*)	Flesh colour	QTL	[167]
Rainbow trout (*Oncorhynchus mykiss*)	Spawning time	QTL	[178]
Rainbow trout (*Oncorhynchus mykiss*)	Albinism	QTL	[170]
Rainbow trout (*Oncorhynchus mykiss*)	High temperature tolerance	QTL	[252]

Table 1. Growth and performance traits for different fish species.

Crustacean species	Trait	Method	References
Pandad shrimp (*Pandalus latirostris*)		Microsatellite	[253]
Giant freshwater prawn (*Macrobrachium rosenbergii*)	Growth traits	SNP	[160]
Ridgetail white prawn (*Exopalaemon carinicauda*)	Growth traits	Transcriptome	[161]
Kuruma shrimp (*Marsupenaeus japonicas*)	Growth traits	QTL	[157]
Kuruma shrimp (*Marsupenaeus japonicas*)	High temperature tolerance	QTL	[157]
Kuruma shrimp (*Marsupenaeus japonicas*)	Growth traits	AFLP	[158]
Pacific white shrimp (*Litopenaeus vannamei*)	Growth traits	QTL	[147]
Kuruma shrimp (*Marsupenaeus japonicas*)	Total and carapace length	ALFP	[254]
Indian black tiger shrimp (*Penaeus monodon*)	Sex determining loci	QTL	[255]
Pacific white shrimp (*Litopenaeus vannamei*)	Sex determining loci	Microsatellite	[256]
Chinese shrimp (*Fenneropenaeus chinensis*)	Body length	QTL	[159]
Pacific white shrimp (*Litopenaeus vannamei*)	Body weight and length	QTL	[257]
oriental river prawn (*Macrobrachium nipponense*)	Body length	QTL	[162]
Kuruma shrimp (*Marsupenaeus japonicas*)	Body length	QTL	[158]

Table 2. Growth and performance traits for different crustacean species.

Body appearance traits identified include the red body colour excluding normal black pigmentation in tilapia [168], silvery skin with few spots in rainbow trout [169], albinism in rainbow trout [170] and melanization in threespine sticklebacks (*Gasterosteus aculeatus*) [171]. Genetic traits essential for improving production in fish farming include traits for feed conversion ratio [172], robustness [173], maturation timing [174], cold tolerance [163, 175], high temperature tolerance [176] and salinity tolerance. In anadromous species such as Atlantic salmon, genetic traits for smoltification [177], migration and spawning timing [178] have been determined.

5.2. Disease resistance and susceptibility traits

The rapid expansion of aquaculture to become one of the leading sources of protein in the world has brought with it an increase in infectious diseases in aquaculture. To reduce the disease

burden and prevent the use of antibiotics, which have been shown to have adverse environmental effects, there has been a tremendous increase in genomics studies aimed at identifying disease resistance traits in different cultured organisms. And as such, different approaches such as SNP, MTLS, AFLP, RAPD, RFLP and QTL analyses have been used for the identification of disease resistance and susceptibility traits in different aquatic organisms. In the case of fish viral diseases, QTL resistance traits have been generated for grass cap reovirus (GCRV) infection in grass carp [179], nervous necrosis virus (NNV) in seabass [180], viral hemorrhagic septicemia (VHS) in turbot [181] and rainbow trout [182], infectious salmon anaemia (ISAV) virus in Atlantic salmon, lymphocytic disease virus in Japanese flounder [183] and infectious pancreatic necrosis virus (IPNV) in Atlantic salmon [184, 185]. Among these, the QTL for resistance against IPNV has contributed to significantly reducing the IPNV incidence by >80% from 2008 when IPNV resistance fish were introduced in the Norwegian Atlantic salmon industry to 2015 [186]. Bacteria disease for which QTL resistance traits have been identified include coldwater disease in rainbow trout [187], *Aeromonas hydrophila* in rohu (*Labeo rohita*) [188], *Vibrio anguillarum* in Japanese flounder [189], *Flavobacterium psychrophilum* in rainbow trout [190] and pastuerellosis in Gilhead seabream [191]. As for parasitic diseases, QTL resistance traits have been identified for *Gyrodactylus salaris* in Atlantic salmon [192] and Monohenean parasite (*Benedenia seriolae*) in Yellow tail (*Seriola quinqueradiata*) [193].

In shrimps, resistance traits have been identified for white spot syndrome virus (WSSV) in Indian black tiger shrimp (*Penaeus monodon*) [194, 195], Fenneropenaeus (*Penaeus chinensis*), infectious hypodermal and hematopoietic necrosis virus (IHHNV) resistance in shrimp (*Litopenaeus stylirostris*) [196] and taura syndrome resistance in Pacific white shrimp (*P. vannamei*) [197]. Among these, the QTL for resistance against TSV has contributed to significant reduction of the disease prevalence in shrimps by generating pathogen-specific free disease shrimps for us in breeding programmes in aquaculture.

6. Application of epigenetics in aquaculture

The term 'epigenetics' was first coined by Waddington in 1942 and was defined as changes in the phenotype without inducing changes in the genotype [198, 199]. Studies on chemical modification of DNA bases date as far back as 1948 [200] and by the 1970s, the role of DNA methylation in gene regulation was identified [201]. In subsequent years, the link between DNA methylation and gene expression was established [202] paving way to the discovery of therapeutic drugs such as 5-azacytidine used to block DNA methylation [203]. In principle, epigenetic changes are regulated by (i) chemical modifications on DNA cytosine residues resulting in DNA methylation and, (ii) histone protein modifications on DNA [204, 205]. Current advances in HTS have refined genomic analyses to base-pair resolution making it easier to map entire epigenomes of living organisms enabling us to identify biological markers predictive of the outcome of disease infections, reproduction, growth and adaptation to new environments [206]. As a result of these advances, epigenetics studies in aquaculture have tremendously increased in the last decades with the view to identifying biological markers relevant for improving the production of farmed aquatic organisms. Technologies used for epigenetics analyses in aquaculture include (i) RNA-seq in

Medaka [207] and Nile tilapia [208]; (ii) genome-wide methylated DNA immunoprecipitation sequencing (MeDIP-seq) in Nile tilapia [209] and Medaka [207]; (iii) bisulfite sequencing (BS-seq) in smooth tongue sole (*Cynoglossus semilaevis*) [210, 211], rainbow trout [212] and Nile tilapia [208]; (iv) genetic linkage map analysis using simple sequence length polymorphisms (SSLPs) in medaka [213, 214]; (v) methylation sensitivity amplified polymorphism (MSAP) in Atlantic salmon [18], grass carp [215], brown trout [17], sea urchin (*Glyptocidaris crenularis*) [216] and sea cucumber (*Apostichopus japonicas*) [217]; (vi) 5-methylcytosine immunolocation in sea lamprey (*Petromyzon marinus*) [218]; (vii) restriction endonuclease hydrolysis of DNA using methylation enzymes in Zebrafish [219] and (viii) bisulfite sequencing PCR in Pacific Oyster (*Crassostrea gigas*) [220] and grass carp [221]. As shown in **Table 3**, epigenetics studies carried out this far include studies on reproduction, growth and adaptation traits. In the case of Atlantic salmon, which is one of the most widely studied species, epigenetic studies have been carried out at different stages of the production cycle as shown in **Figure 2**.

6.1. Embryogenesis and reproduction traits

Embryogenesis and reproduction traits determined by epigenetic analyses in aquatic organisms include sexual dimorphism, embryo development, control of gonadal aromatase and male meiosis [208, 222, 223]. Mhanni and McGowan [219] examined the methylation patterns of the zebrafish genome during early embryogenesis and showed that parental genetic contributions to the zygote were differently methylated with the sperm being more hypermethylated than the oocyte genome. However, immediately after fertilization there was a significant decrease in the embryonic genome methylation, but increased rapidly as the embryo developed to normal levels by the gastrulation stage. These observations are consistent with those seen in mouse [224] suggesting that embryo demethylation/re-methylation is conserved across the vertebrate taxa as of part embryogenesis. As for reproduction traits, Wan et al. [208] found several differentially methylated regions (DMRs) on tilapia chromosomal DNA linked to sexual dimorphism in which the males had high methylation levels after prolonged exposure to high temperature conditions. Similarly, Navarro-Martín et al. [222, 223] showed that European seabass juvenile males had double DNA methylation levels than females in the promoter region of gonadal aromatase, the enzyme that converts androgens to estrogens suggesting that methylation levels on gonadal aromatase were predictive of sex determination. Other fish species for which DNA methylation of aromatase has been linked to sex determination include medaka [225] and Japanese flounder (*Paralichthys olivaceus*) [226]. In crustacean, Gómez et al. [227] analysed the post-translational histone modifications in the testis of *Daphnia magna* and identified cytological markers linked to meiosis progression and the silencing of unsynapsed chromatin. Put together, these studies show that DNA methylation and histone modification can induce reproduction and embryogenesis changes in different aquatic organisms.

6.2. Growth and productivity traits

Epigenetic factors associated with growth and productivity identified in aquatic organisms include early maturation, regulation of muscle growth and disease resistance. Early maturation in Atlantic salmon has emerged to be an interesting topic because prior to migration,

Aquatic organism	Epigenetic trait	References
Zebrafish (*Danio rerio*)	Carcinogenesis	[258]
Zebrafish (*Danio rerio*)	Embryo development	[219]
Zebrafish (*Danio rerio*)	Embryonic cardiogenesis	[259]
Medaka (*Oryzias latipes*)	Excision of ToL2 transposal	[260]
Medaka (*Oryzias latipes*)	Control of cardiomyocyte production in response to stress	[214]
Medaka (*Oryzias latipes*)	Hypoxia and transgenerational reproduction impairment	[207]
Nile tilapia (*Oreochromis niloticus*)	High temperature induced masculinization of skeletal muscles	[209]
Nile tilapia (*Oreochromis niloticus*)	Sexual dimorphism	[208]
Atlantic salmon (*Salmo salar* L.)	Early maturation	[18]
European seabass (*Dicentrarchus labrax*)	Temperature dependent sex ratio shift	[222, 223]
Tongue sole (Cynoglossidae)	Sex reversal	[210, 211]
Senegalese sole (*Solea senegalensis*)	Thermal epigenetic regulation of muscle growth	[261]
European eel (*Anguillarum anguillarum*)	Low cadmium exposure	[232]
European eel (*Anguillarum anguillarum*)	Abnormal ovarian DNA methylation-gonadal	[262]
Red eared slider turtle (*Trachemys scripta elegans*)	Control of gonadal aromatase	[263]
Daphnia magna	Male meiosis	[227]
Pacific oyster (*Crassostrea gigas*)	Growth	[220]
Rainbow trout (*Oncorhynchus mykiss*)	Glucose intolerance	[230]
Rainbow trout (*Oncorhynchus mykiss*)	Migration-related phenotypic divergence	[212]
Atlantic Cod (*Gadus morhua* L.)	Photoperiod influence	[228, 229]
Grass carp (*Ctenopharyngodon idella)*	Individual variations	[215]
Grass carp (*Ctenopharyngodon idella)*	Resistance against grass reovirus	[221]

Table 3. Epigenetics application in aquatic organisms.

parr can reach sexual maturity and successfully fertilize adult females. Up to 60% of total paternity in wild populations has been attributed to these precocious male parr or 'sneakers'. To determine the underlying causes of early sexual maturation in parr, Morán and Pérez-Figueroa [18] compared genetic and epigenetic differences of two populations of parr and mature fish originating from two different rivers and found no genetic difference between

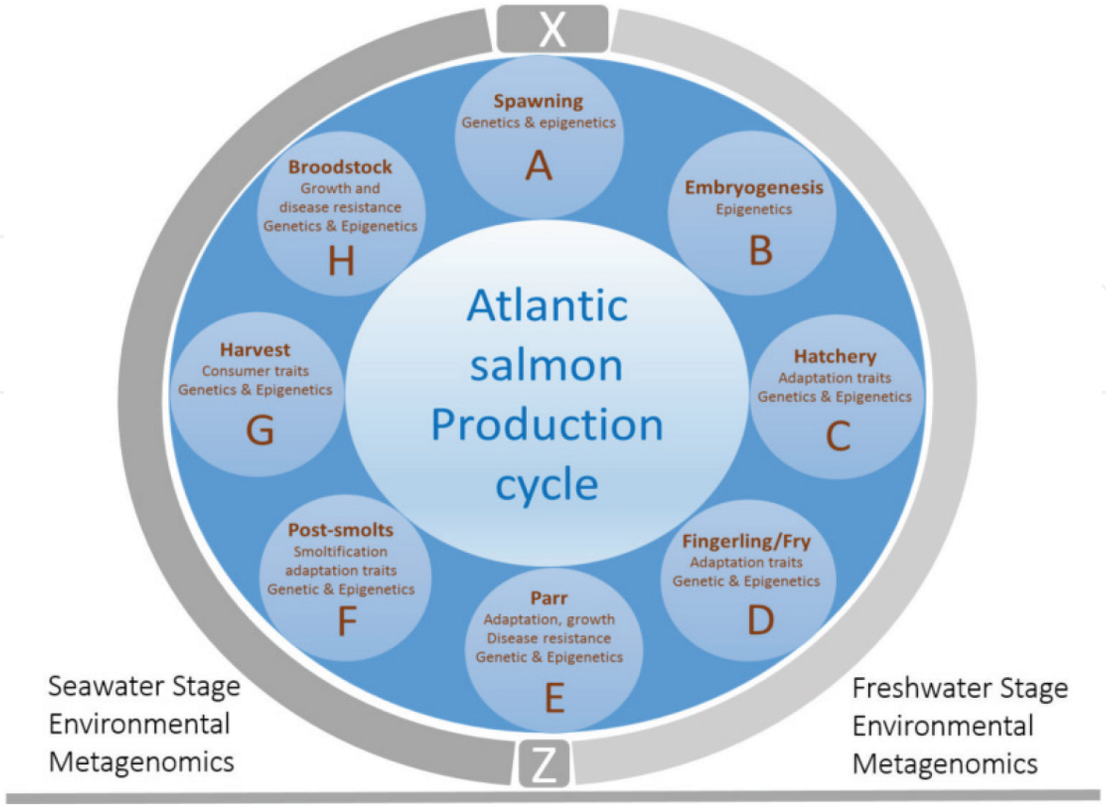


Figure 2. The cycle shows the use of different aspects of functional genomics to improve the production of Atlantic salmon at different stages of the production-cycle. Note that genetics and epigenetics studies are focused on identifying important traits in fish while metagenomics studies are mostly focused on environmental identification of infectious pathogens. Fish from different growth stages are also evaluated for the mucosal microbiota investigations using metagenomics analyses. Nutrigenomics is mostly applied at the outgrower stage. Growth stages are depicted from spawning (A), embryogenesis (B), hatching (C), fingerlings and fry stage (D), Parr stage (E), post-smolts (F), outgrower stage (G) and broodstock (H). Nutrigenomics are after through the feeding stages while the timing of most vaccinations is the parr (D) stage in order to enable fish develop protective antibodies by the post-smolt (E) stage and outgrower stage when they are most vulnerable to stress-related infectious diseases. (X): Depicts the migration of adult fish from seawater into freshwater for spawning. (Z): depicts migration from freshwater to seawater at the parr stage.

parr and mature fish. However, epigenetic analysis showed significant single-locus variations in the gonads followed by the brain and liver between parr and mature fish suggesting that early maturation in Atlantic salmon parr was mediated by epigenetic processes and not genetic differences. As for disease resistance, Shang et al. [221] showed that CpA/CpG methylation of grass carp *Ctenopharyngodon idella* melanoma differentiation associated gene 5 (MDA5) (CiMDA5) was tightly associated with resistance against GCRV. In their findings, they found CpA/CpG methylation sites in the CiMDA5 genome that consisted of putative densely methylated elements (DMEs) that were significantly higher in GCRV susceptible fish than in the resistant fish. In terms of muscle growth, Giannetto et al. [228] found a correlation between DNA (cytosine-5)-methyltransferases (DNMTs) increase in fast muscle with prolonged exposure to light indicating that photoperiod influence may be involved in the DNMTs regulation of muscle growth in Atlantic cod. Similarly, Nagasawa et al. [229] found high histone methyltransferases levels of the mixed-lineage leukaemia (MLL) gene in fast muscle of Atlantic cod subjected to prolonged light exposure, which corresponded with

increase in mRNA expression of myogenic regulatory factors (*Myog* and *Myf-5*) and *Pax7* in fast muscle. Overall, these studies show that DNA methylation and histone modification of chromosomal DNA play an important role in regulating muscle growth, disease resistance and sex maturation in fish.

6.3. Adaption epigenetic traits

Epigenetic factors shown to induce adaptation changes in cultured aquatic organisms include nutrition, migration, salinity and photoperiod exposure. Several nutritional studies have shown that rainbow trout displays persistent hyperglycaemia when fed high carbohydrate (HighCHO) diets. To underpin the underlying causes, Marandel et al. [230] examined the liver of rainbow trout fed HighCHO diets and found global DNA hypomethylation and hypoacetylation of histone H3K9 resembling hyperglycaemic and diabetes conditions in zebrafish and mammals. They also showed that *g6pcb2* ohnologs that encode the glucose-6-phosphatase (G6pc) enzyme involved in gluconeogenesis catalysis were hypomethylated at specific CpG sites indicating that the hepatic epigenetic landscape of rainbow trout can be affected by dietary carbohydrates. As for migration traits, Baerwald et al. [212] identified several DMRs between migratory smolts and resident rainbow trout juveniles in which most DMRs encoded proteins associated with migration showing that epigenetic variations were linked to migration traits in anadromous fish. Their findings were in concordance with Morán et al. [17] who found genome-wide methylation differences between hatchery reared and seawater brown trout. In addition, Morán et al. [17] showed that salt diets used during the seawater phase triggered genome-wide methylation changes when administered in freshwater reared trout indicating that DNA methylation could play a vital role in enabling anadromous fish acclimatize to seawater after transfer from freshwater. DNA methylation and histone modification have also been associated with adaptation changes induced by adverse environmental conditions as shown in Nile tilapia exposed to industrial pollutions [231], eels to cadmium exposure [232], sea urchin (*G. crenularis*) exposure to perfluoroctane sulfonate (PFOS) [216] and the three-spine stickleback (*G. aculeatus*) hexabromocyclododecane (HBCD) exposed to 17-β oestradiol (E_2) and 5-aza 2′ deoxycytidine (5AdC) pollutants [233]. In summary, these studies demonstrate that DNA methylation and histone modification contribute to nutritional, environmental and photoperiod adaptation in different aquatic organisms and that these factors could have an influence on improving production in aquaculture.

7. Whole genome sequencing of aquatic organisms

Although teleost fish are the largest known vertebrate group with more than 27,000 species [8], they account for a small proportion of vertebrate species whose whole genomes have been fully sequenced and characterized. The pufferfish genome is one of the earliest fish genome to be sequenced and characterized by 2002 [234], which raised interests to sequence the genomes of other fish species. The zebrafish (*Danio rerio*) whole genome sequencing project was started by Welcome Trust Sanger Institute in 2001 [235] while the Medaka genome was sequenced in 2007 [236]. Thus, Zebrafish and medaka are not only among the earliest

fish species to have their genomes sequenced and characterized, but they have attracted the highest research in genomic studies among teleost species. Their genomes have been widely used for comparative analyses as model species [235, 237–239]. Sequence analyses of the Atlantic cod genome in 2011 using the whole genome shotgun 454 pyrosequencing technology showed that this fish species lacks the major histocompatibility (MHC) II genes, which are compensated with expansion of the MHC-I and specific adaption of toll-like receptor genes demonstrating that whole genome sequencing can be used to elucidate evolutionary differences in the vertebrate taxa [240]. As shown in **Table 4**, there has been a spontaneous increase in the number of fish species whose genomes have characterized since the discovery of HTS technologies in recent years. Sequencing of other aquatic organism genomes is going on and it is anticipated that as HTS becomes cheaper, more sequences of aquatic organisms will become readily available for more advanced functional genomics research in aquaculture.

Common name	Scientific name	Year Published	Reference
Atlantic salmon	*Salmon salar* L.	2016	[264]
Atlantic cod	*Gadus morhua*	2011	[240]
Asian arowana	*Scleropages formosus*	2015	[8]
Medaka	*Oryzias latipes*	2007	[236]
Nile tilapia	*Oreochromis niloticus*	2015	[7]
Platyfish	*Xiphophorus maculatus*	2013	[265, 266]
Puffer fish	*Takifugu rubripes*	2002	[234]
Puffer fish	*Tetraodon nigroviridis*	2004	[267]
Three-spined stickleback	*Gasterosteus aculeatus*	2012	[268]
Rainbow trout	*Oncorhynchus mykiss*	2014/2016	[269, 270]
Killifish	*Nothobranchius furzeri*	2015	[271, 272]
Pearl oyster	*Pinctada fucata*	2012	[273]

Table 4. Whole genome sequencing of aquatic organisms.

8. Conclusions

In this chapter, we have shown that HTS has contributed to the rapid discovery of novel pathogens in aquaculture using metagenomics, which has significantly contributed in enhancing our ability to develop rationale disease control strategies unlike in the past when it took long from the first report of a clinical disease to identification of a novel pathogen. Moreover, metagenomics enable us to identify and monitor microbial communities found in different ecosystems

used in aquaculture. It has also proved to be an important tool able to map mucosal microbiota of different aquatic organisms. In vaccine production, genomics studies are being used to identify cross-neutralizing antigens able to confer protection across variant strains of the same pathogens. In genetics and epigenetics, several genomics traits have been identified that currently contributing to the improvement of production in aquaculture. Nutrigenomics have not only enhanced our understanding of the genetic markers for enteropathy and other nutritional diseases, but they have also highlighted our ability to formulate diets able to maintain stable GALT homeostasis in the gut. And as shown from the example of the Atlantic salmon production cycle in **Figure 2**, it is evident that functional genomics are used at different production stages of aquatic organisms to improve the overall production in aquaculture. Hence, genomics studies are not only useful at elucidating host-pathogen interactions [13-15], but they also serve as optimization tools for improving the quality and quantity of aquaculture products.

9. Future perspective

As HTS technologies become cheaper, it is anticipated that more genomes for different aquatic organisms will characterized and that this shall pave to a better understanding of the genome duplication seen in some fish species. The use of HTS technologies in pathogen discovery and microbiota inhabiting mucosal surfaces of different aquatic organisms is expected to pave way into timely design of rational disease control strategies. Hence, in future generations, we shall not only sequence whole genomes of all aquatic organisms, but we expect to provide a better understanding of the evolutionary aspects of the vertebrate taxa as well as providing new insight into host-pathogen interaction mechanisms at protein-protein level. It is our perception that current HTS studies are building a strong foundation for more advanced functional genomics developments in the future.

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