REVIEW NOTE ON THE APPLICATION OF METAGENOMICS IN EMERGING AQUACULTURE SYSTEMS AND AQUATIC ANIMAL HEALTH MANAGEMENT

[*1]Kaviarasu, D. and [2]Sudhan, C.

[1]Department of Fish Pathology and Health Management, School of Aquaculture, Fisheries College and Research Institute, Tamil Nadu Fisheries University, Thoothukudi, Tamil Nadu, India.

[2]Department of Fisheries Biology and Resource Management, School of Fisheries Resource and Environment Management, Fisheries College and Research Institute, Tamil Nadu Fisheries University, Thoothukudi, Tamil Nadu, India.

Abstract: Microbes are the abundant biological entities found in the biosphere. Identification and measurement of microorganisms (including viruses, bacteria, fungi and protists) cannot be completely achieved due to limitations in culture methods. Over the past decade, "Metagenomics," which are culture-independent genomics analysis of microbes, has been developed to overcome those problems. Metagenomics are the study of the collective genomes of the members of a microbial community. This paper would bring a basic knowledge about the metagenomics, related technology applied in the field of fisheries and their application of modern genomics techniques in aquatic animal health management, aquaculture dealing with microbial diversity, microcosms, antibiotic resistance genes, detection of pathogens microbial communities forming bioflocs and Probiotics.

Key Words: Metagenomics, Bioflocs, Probiotics, Technology, Fisheries.

1. INTRODUCTION:

Microbes were the abundant biological entities found in the biosphere. Identification and measurement of microorganisms (including viruses, bacteria, fungi and protists) cannot be completely achieved due to limitations in culturing methods. Micro-organism communities were important in the functioning of all ecosystems, but the unculturable microorganisms and their role in natural ecosystems were unclear. Metagenomics was based on the genomic analysis of microbial DNA directly from the communities present in samples such as soil, water or faeces. The use of traditional microbiological culturing methods for the study of microbes has limited success. It has been estimated that 99% of the microbes cannot be cultivated easily. Different problems have been faced by researchers during attempts to culture some microbes. Metagenomics were new and exciting field of molecular biology that was likely to grow into a standard technique for understanding biological diversity **(Ghazanfar *et al.*, 2010)**.

Over the past decade, "Metagenomics," which were culture-independent genomics analysis of microbes, has been developed to overcome these difficulties. It involves cloning and analyzing the genomes without culturing the organisms in the community thereby offering the opportunity to describe the planet's diverse microbial inhabitants, many of which cannot yet be cultured. Metagenomics also called as community genomics, environmental genomics and population genomics **(Sabree *et al.*, 2009).**

The Term coined by Jo Handelsman and others in the University of Wisconsin, Department of Plant Pathology in 1982 **(Handelsman, 2004).** The application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments bypassing the need for isolation and laboratory cultivation of individual species study of collective genomes of the members of a microbial community **(Ghazanfar *et al.*, 2010),** starting from the first cloning of DNA directly from environmental samples in a phage vector and that subsequently lead to several studies starting from the first cloning of DNA directly from environmental samples in a phage vector culminating in the

direct random shot-gun sequencing of environmental DNA (**Neelakanta and Sultana, 2013**). At first non-cultured microflora and ancient DNA investigations were the prime targets of metagenomic studies. Nowadays this technology was applied in the study of an array of microbial diversities like deep sea aquatic micro-flora, soil microbes and gastrointestinal tract ecosystems of animals and human. Studies have revealed that only 0.001-0.1% of the total microbes in sea water, 0.25% in freshwater, 0.25% in sediments and only 0.3% of soil microorganisms could be cultivable in vitro. The current metagenomic studies have largely progressed due to the construction of efficient gene cloning vectors like bacterial artificial chromosomes (BACs) or cosmids which allow cloning and expression of larger and complex DNA segments or genes and the development of methods for generation and analysis of the data.

2. STEPS INVOLVED IN METAGENOMICS:

2.1. Sampling and nucleic acids extraction: In metagenomics, process the samples could be processed from any environment, soil or habitat ecosystem. Although various kits were commercially available for DNA isolation from environmental samples, many laboratories have developed their own methods with the aim of optimizing extraction and reducing bias caused by unequal lysis of different members of the soil microbial community. There were two types of extraction techniques: (1) Direct, in situ, extraction where the cells were lysed in the soil sample and then the DNA was recovered; and (2) Indirect extraction techniques, where the cells were removed from the soil and then lysed for DNA recovery **(Schmeisser *et al.,* 2007).** Soil was found to be particular complex matrix containing many substances, such as humic acids, which could be co-extracted during DNA isolation. Removal of humic acids was essential before the DNA could processed further. For this purpose, a range of DNA purification techniques has been developed. Sephadex G-200 spin columns have proven to be one of the best ways to remove contaminants from soil DNA **(Miller *et al.,* 1999).** Recently, a pulse field electrophoresis procedure using a two-phase agarose gel, with one phase containing polyvinylpyrrolidone (PVPP), was developed for removal of humics **(Quaiser *et al.*, 2002).**

2.2. Construction of a metagenomic library: DNA isolation and purification was followed by the construction of DNA libraries in suitable cloning vectors and host strains. The classical approach includes the construction of small insert libraries (<10 kb) in a standard sequencing vector and in *Escherichia coli* as a host strain. However, small insert libraries do not allow detection of large gene clusters or operons. To circumvent this limitation researchers have been employing large insert libraries such as cosmid DNA libraries with insert sizes ranging from 25-35 kb or Bacterial Artificial Chromosome (BAC) libraries with insert up to 200 kb. Additionally, the construction of fosmid with inserts of 40 kb of foreign DNA has been reported. *E. coli* was still the preferred host for the cloning and expression of any metagenome-derived genes and only very recently have other hosts such as *Streptomyces lividans* been employed to identify genes involved in the biosynthesis of novel antibiotics **(Courtois *et al.*, 2003).** Metagenomic libraries were also being developed in other Gram-negative hosts by several laboratories, and these will become available soon.

2.3. Analysis of metagenomic libraries: Two methods were used for the analysis of genetic material of metagenomic library.

4.3.1. Sequence-based metagenomics: It provides information on the distribution of functions in a community, linkage of traits, genomic organization and horizontal gene transfer. Approaches typically involve either sequencing of random clones to accumulate vast stores of sequence information or identification of clones based on methods that detect a particular sequence. With both of these approaches, phylogenetic markers were sought on the clone of interest to link cloned sequences with the probable origin of the DNA.

4.3.2. Function-based analysis: Function-based analysis enables identification of new enzymes, antibiotics or other reagents in libraries from diverse environments. Approaches include: (a)

Heterologous expression, in which clones that express the desired functions were identified. An important limitation to heterologous expression was that the domesticated host bacterium must be able to express (transcribe and translate) the genes for the products to be detected; (b) Selections provide the most powerful approach to finding rare clones. Examples of selectable characteristics include antibiotic resistance and metal resistance.

3. POTENTIAL USES OF METAGENOMICS IN AQUATIC ANIMAL HEALTH MANAGEMENT:

Metagenomics were a relative recent genomics sub discipline that has emerged as a promising scientific tool to analyze the complex genomes contained within microbial communities. Metagenomics approaches in aquaculture used to study of several systems like microbial diversity, microbial roles in microcosms, antibiotic resistance genes, novel and potential pathogens, microbial communities forming bioflocs, probiotics and other applications. These technologies used to highlight the microbial diversity and dynamics of the culture systems in aquaculture sector.

4. POTENTIAL USES OF METAGENOMICS IN THE FIELD OF AQUACULTURE:

In aquaculture, nitrogenous and phosphorous metabolites and organic matter were abundant making aquaculture an ideal media for the proliferation of microorganisms for this reason it is assumed that the diversity of microbial DNA within aquaculture facilities could be even greater. The above scenario reveals that at present the microbiological information of aquaculture perhaps represents only a very small part of an entire universe. The complete diversity and hypothetical roles of un-culturable microorganisms were difficult if not impossible to prove without genomic techniques these questions may be answered by metagenomics and functional genomics coupled with chemical ecology.

Microbial studies in aquaculture were focused on understanding the symbiotic and antagonist interrelationships among microbes in relation with eukaryotes such as fish, crustacean and molluscs. In this regard, metagenomics could provide a deeper insight into these relationships by performing associations of the information revealed by the extracted DNA with particular host organisms or ecosystems. For instance intracellular pathogenic bacteria have been difficult to isolate because some of them were intracellular obligate microorganisms that could be cultured in semi-aqueous or cell culture media. New sequencing and bioinformatics technologies make it possible not only to investigate the diversity of intracellular bacteria but also to elucidate relevant genomic information from such communities.

4.1. Microbial diversity: Metagenomics could provide additional evidence that enables the understanding of the microbial diversity that thrives within aquaculture facilities. For instance **Krishnani *et al.*, (2010a and b)** described the diversity of sulphur-oxidizing bacteria in a green water system of coastal aquaculture the authors documented the unexpected presence of *Pseudoxanthomonas* sp. (a sulphur chemolithotrophic c-proteobacterium). These results explained the maintenance of sulphur at the prescribed safe levels in that area despite the aquacultural activity.

4.2. Role of microbes and their actions in microcosms: Genes were considered to be the basic functional units in the genome of any organism, constituting transcriptional units, operons and networks. Gene-finding algorithms have been designed to identify open reading frames within single-species genomes, but according to **Wooley *et al.*, (2010),** that information was unavailable or merely not accessible for metagenomics because of the incomplete and fragmentary type of metagenomics data. However, **Mavromatis *et al.*, (2007)** reported that gene predictions based on the assemblies of low-complexity metagenomics data sets could be as accurate as 90%, with an average of 85% accuracy for high-complexity sets. Additionally, Blasting was a common practice used to search for genes with known homologs that could also be used to identify the existence of gene family members in a metagenome **(Azad and Borodovsky, 2004)**. One disadvantage of BLAST was that it was not useful for finding new families and novel genes that have no homologs in known

databases **(Wooley *et al*., 2010).** As stated previously the information of microbial diversity and dynamics in aquaculture was not completely understood. Therefore aquaculture was an example of the importance of performing diversity and random shotgun studies of genes. Once the information was available, researchers will be able to provide deeper insights into the dynamics of microbial populations formed in culture units such as tanks, ponds, raceways, jails and open sea jails.

Therefore more data will be accessible and enable us to reach a better understanding not only concerning the common microbial communities formed in ponds (or other culture units) but also concerning the occurrence of imbalances in microbial communities (particularly in open aquaculture systems taking water from oceans and rivers) the scenarios that cause pathogen outbreaks, the function and relationships of probiotics, the enhancement of antibiotic resistance in bacteria, and the role of aerobic bacteria as food chain links. Many investigations have focused on the study of microorganisms as pathogens, probiotics, food sources and sentinels **(Aguilera-Rivera *et al*., 2014)** however, information regarding their unculturable microbial neighbours (i.e. diversity, relationships and genes) is still scarce.

Many metabolic processes triggered by particular genes have important relevance; however, in aquaculture, there were some genes that may have more importance than the rest. Aquaculture generates millions of tons of waste each year, of which considerable parts were nitrogenous, sulphurous, phosphorous and carbonous compounds **(Wang *et al*., 2012);** these wastes sometimes toxic and rebase the nutrient loading capacity of the ecosystems. Thus the study of genes and operons encoding for proteins involved in sensing, uptake and utilization of particular metabolites commonly observed in aquaculture has become of paramount importance because the capacity and biochemical pathways of a given microbial community to recycle nutrients will then be understood. Moreover these genes also be used for bioremediation or recirculation purposes.

4.3. Antibiotic resistance genes: The use of antibiotics in aquaculture was perhaps the most popular therapeutic strategy for the treatment of infectious diseases caused by bacteria but its success was compromised by the flowering of tolerance or resistance to a particular compound from the time of its first application. **Schmieder and Edwards (2012)** reported at least four well-known mechanisms that contribute to antibiotic resistance in bacteria: (i) the inactivation or modification of the antibiotic, (ii) an alteration in the target site of the antibiotic that reduces its binding capacity, (iii) the modification of metabolic pathways to circumvent the antibiotic effect and (iv) the reduced intracellular antibiotic accumulation by decreasing the permeability and/or increasing the active efflux of the antibiotic. Bacteria could acquire resistance to antibiotics though a variety of mechanisms including the modification of existing genes horizontal gene transfer (mobile genetic elements) and the presence of low levels of particular antibiotics in the environment that may represent a gene transfer promoter. Conventional methods for the detection of antibiotic resistance usually include using growth inhibition assays in broth or agar disc diffusion, in which the minimal inhibitory concentration (MIC) of particular antibiotics could be estimated for each bacterial isolate. One of the limitations of this procedure lies in the fact that only a few bacterial isolates could be studied at a time contrasting with the millions of bacterial species that could be present within aquaculture facilities and the effluent-receiving ecosystems that would be under-represented **(Moura *et al*., 2010).** Another problem associated with culture-based systems was the culturing time which may take from 1 to 2 days for fast-growing bacteria to several weeks for slow-growing species. While novel methods involving quantitative PCR and microarray technologies have been developed these only serve to detect the presence of specific well-studied genes related to antibiotic resistance and to report results within hours **(Perreten *et al*., 2005).**

4.4. Detection of pathogens and their multidisciplinary functions: To date, several animal and plant pathogens have been and were still being reported. The dissemination of diseases was favored by

features of viral agents such as rapid mutation and vertical/horizontal transmission leading to the occurrence of epizooties. Viral species exhibit unique infection, transporting and persistence processes. The identification of viral mechanisms may contribute to the rapid identification of pathogenic species and mutations that represent useful information for diagnosis, prevention and the development of treatments. Unfortunately, the identification of pathogens as well as the existing diagnostic methods was circumscribed by an incomplete picture of the enormous viral diversity and the limitations of traditional detection methods. Traditionally, viral pathogens were detected in monolayer cultures that may exhibit cytopathic effects or through antibody neutralization assays. However, antibody neutralization approaches depend upon the availability of antiserum, and most of the viral species were not readily culturable under laboratory conditions **(Wang *et al.*, 2002),** which increases the difficulty of identification and discovery of new pathogens as well as their basic study. During the last decades, molecular-based methods such as PCR have been implemented for the study and detection of un-culturable and/or non-isolated viruses; however, PCR-based methods require previous genomic information, which usually precludes the detection and study of emerging viruses **(Gao and Moore 1996).** Shotgun metagenomics of clinical or random environmental samples represents a promising alternative that circumvents the limitations of the traditional methods. Although this technique has been typically used to study genomic diversity, it could also be useful for the identification of viral pathogens in clinical and environmental samples.

Studies of viruses using metagenomic approaches have been recently encouraged because of the quality and quantity of genomic information obtained with next-generation sequencing; although Sanger sequencing provides significantly less information, it has also been used as an identification tool. Metagenomics has demonstrated a better performance in terms of efficiency and precision in the detection of multiple genomes compared to other methods such as PCR or microarrays. Viral metagenomics exceeds the coverage and efficiency of any other method used for random identification; moreover, as stated above, metagenomics does not require previous genomic information about any particular pathogen, expensive (and usually inefficient) culture media or antibody laboratory tests because the identification of multiple pathogens could be performed in a single sample. Agro-industrial activities could also be greatly benefit due to the potential of viral metagenomics to serve as a sentinel tool for the rapid detection of known, new or potential pathogens and to help elucidate some of their invasion and reproduction mechanisms.

4.5. Microbial communities in relation with formation of bioflocs: The use of microbial biofilms and bioflocs as direct food sources has been exhibited a vigorous growth accompanied by successful results over the last decade. For example, yield production of fish and shrimp could doubled when bioflocs were combined with formulated feed because they were a rapid growing source of not only protein but also vitamins and lipids (depending on their composition). These characteristics allow biofloc users to increase the intensification of the activity (super intensive and hyper intensive aquaculture). For these reasons, biofloc technology (BFT) was worthy of a special section. For the last four decades, aquaculture has used mostly artificial feed, with 70–80% remaining in the water column or sediments. This situation results in not only a waste of feed and money but also the production of toxic residues that affect the growth rates of the cultured species and limit the intensification of the activity (**Avnimelech, 2012).** Thus, BFT has arisen as a successful strategy to take aquaculture intensification and sustainability to the next level. BFT takes advantage of the accumulation of organic residues and the limited water exchange. Then, mixing and aeration were performed to provide the ideal conditions for the proliferation of aerobic heterotrophic bacteria; fish/crustaceans eat the bacteria, and the nutrients were recycled.

4.6. Application of probiotics: The use of probiotics in humans has been a success. Probiotics that contribute in the reconstruction of the native intestinal microflora of the host when they were added

into feedstuffs thus, when consumed in appropriate amounts, probiotics may provide health benefits for the host. Aquaculture has been one of the many agro-industrial activities that have adopted the use of probiotics for different purposes. The use of probiotics in aquaculture should consider the microbial consortia with deep, extensive and constant interaction with the host and the environment to function as the intestinal microbiota. Therefore, an adequate probiotic definition for aquaculture could be described as a live microbial complement providing health benefits to the host through the modification of its intrinsic microbial community or by the adjustment of the environmental microbial community, optimizing the use nutrients and/or the immune response, and/or improving the environmental quality at microcosms level (i.e. culture units, ponds, tanks and raceways).

The complexity of microbial communities in terms of diversity and functionality may be as huge as those reported in BFT systems and sediments. Metagenomics represents the same useful tool for monitoring microbial communities in aquaculture systems using probiotics. This strategy might provide an insight into understanding the microbial dynamics (synergisms and antagonisms) occurring within the culture systems and identifying the microbial consortia necessary for the well-being and protection of the cultured organisms.

Vibrio sp., *Bacillus* sp., lactic acid bacteria and microalgae have been successfully used in fish and crustacean aquaculture to improve growth and survival and for the eradication of pathogens **(Austin *et al.*, 1992; Austin *et al.*, 1995; Gildberg *et al.*, 1997; Rengpipat *et al.*, 1998).** Marine water, ocean sediments and the gastrointestinal tracts of marine species have been used as probiotic sources for aquaculture, particularly those able to produce substances that inhibit the proliferation of pathogens and thereby maintain biosecured microcosms.

Functional metagenomics could be used to discover genes encoding novel bioactive molecules and proteins with particular antibiotic activity or enzymes aiding in the digestion process. Some other probiotics may indirectly inhibit the movement of bacteria throughout the gut wall (translocation), improve mucus function by increasing the production of midgut–intestine immune molecules or modulate the inflammatory/immune response. For instance, these mechanisms have been inferred by detecting the activation of pattern recognition receptors (PRPs) such as Toll-like receptors (TLRs) that play key roles in the innate immunesystem through the activation of immune cell responses; these responses may include the secretion of antimicrobial peptides (defensines and chemokines) through the epithelial cells **(Sherman *et al.*, 2009; Quigley 2010).**

5. OTHER APPLICATIONS:

Bacteria have also been used for the bioremediation of aquaculture effluents; metagenomic libraries for particular biodegradation genes could be constructed, amplified and screened. This technique has been tested by cloning genes into bacteria that were then incubated in media containing high concentrations of toxic compounds **(George *et al.*, 2010).** Environmental communities exposed for long periods to xenobiotics were expected to be enriched in biodegradation genes. Rich nutrient sediments generated in aquaculture and in the effluent-receiving ecosystems possess the conditions necessary for the proliferation of entire microbial communities that could be a source of novel genes with usable functions from a biotechnological standpoint. This technique could provide new molecules with diverse functions that might represent medical solutions and/or economic strategies **(Lorenz and Eck, 2005).** Both novel small-molecule antibiotics and new antibacterial proteins have been identified using metagenomic approaches. Genes encoding bioactive compound such as the antibacterially active pigments violacein, indigo and turbomycins and cyclic peptides such as nocardamine have been recovered from soil libraries **(Banik and Brady, 2010).**

Finally, aquaculture would be diverse in nature based on its production scale and not only in terms of the species, but in terms of water type (freshwater, brackishwater, sea water), density (extensive to hyperintensive) and system technology (from outdoor earthen ponds to mariculture cages

or indoor recirculating systems), but metagenomics has applications for all those above mentioned combinations. Regarding epizooties, most of the research was usually focused on the biology of the pathogen, but there was no information about the effect of the pathogen in the microbial diversity of the host, or about any possible relationship between particular modifications in the microbial diversity of the environment and/or the host that favours the proliferation of the pathogen. On the other hand, there were no studies about the microbial diversity (based on metagenomic approach) contained in the effluents that were discharged by farms to the environment or the changes caused on the natural biota. These priorities in metagenomic applications were not rule of thumb and can be changed depending upon the different research questions of particular groups of scientists.

Certain microorganisms able to degrade waste products, to make new drugs for medical applications, to produce environmentally friendly plastics, or even make some of the food we eat. Gut microbiota also important for the host animals like nutrition, immune system, metabolic function, physiology and growth. By isolating the DNA from these organisms, it provides an opportunity to optimize these processes and adapt them for use by society. It provides the capacity to effectively characterize the genetic diversity present water, soil and rumen source samples regardless of the availability of laboratory culturing techniques.

6. CONCLUSION:

The emerging technique 'Metagenomics' was based on random shotgun sequencing may provide the opportunity to identify novel bacterial and viral strains before it could arise a problem in the culture system that directly has an impact on reduced production in terms of tons and animal health in terms of disease or mortality. Metagenomics has been under used in aquaculture considering the vast universe of potential applications that may answer scientific questions, solve problems and produce novel products with biotechnological value.

7. REFERENCES:

8. Aguilera-Rivera, D., Prieto-Davo, A., Escalante, K., Chavez, C., Cuzon, G., Gaxiola, G., 2014. Probiotic effect of FLOC on *Vibrios* in the pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*. 424-425, 215-219.
9. Austin, B., Baudet, E., Stobie, M.,1992. Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. *Journal of Fish Diseases*. 15, 55-61.
10. Austin, B., Stuckey, L.F., Robertson, P.A.W., Effendi, I., Griffith, D.R.W., 1995. A probiotic strain of *Vibrio alginolyticus* effective in reducing diseases caused by *Aeromonas salmonicida, Vibrio anguillarum* and *Vibrio ordalii*. *Journal of Fish Diseases*. 18, 93-96.
11. Avnimelech, Y., 2012. Biofloc Technology. World Aquaculture Society, Baton Rouge, LA.
12. Azad, R.K., Borodovsky, M., 2004. Probabilistic methods of identifying genes in prokaryotic genomes: connections to the HMM theory. *Briefing in Bioinformatics*. 5, 118–130.
13. Banik, J.J., Brady, S.F., 2010. Recent application of metagenomic approaches toward the discovery of antimicrobials and other bioactive small molecules. *Current Opinion in Microbiology*. 13, 603–609.
14. Courtois, S., Cappellano, C.M., Ball, M., Francou, F.X., Normand, P., Helynck, G., Martinez, A., Kolvek, S.J., Hopke, J., Osburne, M.S., August, P.R., Nalin, R., Guerineau, M., Jeannin, P., Simonet, P., Pernodet, J.L., 2003. Recombinant environmental libraries provide access to microbial diversity for drug discovery from natural products. *Applied and Environmental Microbiology*. 69, 49–55.
15. Gao, S.J., Moore, P.S., 1996. Molecular approaches to the identification of unculturable infectious agents. *Emerging Infectious Diseases*. 2,159.
16. George, I., Stenuit, B, Agathos, S.., Marco, D., 2010. Application of metagenomics to bioremediation. *Metagenomics: Theory, Methods and Applications*. 1, 119-140.
17. Ghazanfar, S., Azim, A., Ghazanfar, M.A., Iqbal, M., 2010. Metagenomics and its application in soil microbial community studies: biotechnological prospects. *Journal of Animal & Plant Sciences*. 6, 611-622.

18. Gildberg, A., Mikkelsen, H., Sandaker, E., Ringo, E., 1997. Probiotic effect of lactic acid bacteria in the feed on growth and survival of fry of Atlantic cod (*Gadus morhua*). In: *Asia-Pacific Conference on Science and Management of Coastal Environment. Springer*, 123: 279–285.

19. Handelsman, J., 2004. Metagenomics: Application of Genomics to Uncultured Microorganisms. *Microbiology and molecular biology*. 68, 4.

20. Krishnani, K.K., Gopikrishna, G., Pillai, S.M., Gupta, B.P., 2010. Abundance of sulphur-oxidizing bacteria in coastal aquaculture using soxB gene analyses. *Aquaculture Research*. 41, 1290-1301.

21. Krishnani, K.K., Kathiravan, V., Natarajan, M., Kailasam, M., Pillai, S.M., 2010. Diversity of sulfur-oxidizing bacteria in greenwater system of coastal aquaculture. *Applied Biochemistry and Biotechnology*. 162, 1225-1237.

22. Lorenz, P., Eck, J., 2005. Metagenomics and industrial applications. *Nature Reviews Microbiology*. 3, 510-516.

23. Mavromatis, K., Ivanova, N., Barry, K., Shapiro, H., Goltsman, E., McHardy, A.C., 2007. Use of simulated data sets to evaluate the fidelity of metagenomic processing methods. *Nature Methods*. 4, 495-500.

24. Miller, D. N., Bryant, J.E., Madsen, E.L., Ghiorse, W.C., 1999. Evaluation and optimization of DNA extraction and purification procedure for soil and sediment samples. *Applied and Environmental Microbiology*. 65, 4715-4724.

25. Moura, A., Henriques, I., Smalla, K., Correia, A., 2010. Wastewater bacterial communities bring together broad-host range plasmids, integrons and a wide diversity of uncharacterized gene cassettes. *Research in Microbiology*. 161, 58-66.

26. Neelakanta, G., Sultana, H., 2013. The Use of Metagenomic Approaches to Analyze changes in Microbial communities. *Microbiology Insights*. 6, 37-48.

27. Perreten, V., Vorlet-Fawer, L., Slickers, P., Ehricht, R., Kuhnert, P., Frey, J., 2005. Microarray-based detection of 90 antibiotic resistance genes of gram-positive bacteria. *Journal of Clinical Microbiology*. 43, 2291-2302.

28. Quaiser, A., Ochsenreiter, T., Klenk, H.P., Kletzin, A., Treusch, A.H., Meurer, G., Eck, J., Sensen, C.W., Schleper, C., 2002. First insight into the genome of an uncultivated crenarchaeote from soil. *Environmental Microbiology*. 4, 603-611.

29. Quigley, E.M.M., 2010. Prebiotics and probiotics; modifying and mining the microbiota. *Pharmacological Research*. 61. 213-218.

30. Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S., Menasveta, P., 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture*. 167, 301-313.

31. Sabree, Z.L., Rondon, M.R., Handelsman, J., 2009. Metagenomics. In *Encyclopedia of Microbiology*. 3rd edn, pp. 622-632. Academic Press.

32. Schmeisser, C., Helen, S., Wolfgang, R.S., 2007. Metagenomics, biotechnology with nonculturable microbes. *Appl Microbiol Biotechnol*. 75, 955-962.

33. Schmieder, R., Edwards, R., 2012. Insights into antibiotic resistance through metagenomic approaches. *Future Microbiology*. 7, 73-89.

34. Sherman, P.M., Ossa, J.C., Johnson-Henry, K., 2009. Unraveling mechanisms of action of probiotics. *Nutrition in Clinical Practice*. 24,10-14.

35. Wang, D., Coscoy, L., Zylberberg, M., Avila, P.C., Boushey, H.A., Ganem, D., 2002. Microarray-based detection and genotyping of viral pathogens. *Proceedings of the National Academy of Sciences*. 99, 15687-15692.

36. Wang, X., Olsen, L.M., Reitan, K.I., Olsen, Y., 2012. Discharge of nutrient wastes from salmon farms: environmental effects and potential for integrated multi-trophic aquaculture. *Aquaculture Environment Interanctions*. 2, 267-283.

37. Wooley, J.C., Godzik, A., Friedberg, I., 2010. A primer on metagenomics. *PLoS One*. 6, e1000667. doi:10.1371/journal.pcbi.1000667.