Zymo Spin III-HRC Filter is placed into a new Collection tube and 600 µl Prep Solution is added, then centrifuged at 8,000 xg for 3 minutes. DNA elution was transferred to the Zymo-Spin ™ III-HRC filter which had been prepared in a clean 1.5 ml microcentrifuge tube, then centrifuged at 8,000 xg for 3 minutes.

2.3 Visualization and Measurement of Concentrations, Purity of DNA Isolated

Visualization and measurement of concentration, purity of DNA isolation results are needed to determine the quality and quantity of the sample. Visualization of the results of isolation was done by electrophoresis. The first step is to make 1% agarose gel weighed with 1 gram agarose powder and add 100 ml of TAE 1 × to the Erlenmeyer tube. Then the ingredients are heated in the microwave until the ingredients are evenly mixed. Then 10 ml of the red gel was added and the gel was printed. Then 2 µl Bench Top DNA Ladder 1 kb and 2 µl loading dye were put into the first gel well. A total of 4 µl of 2 µl loading dye insulation product was put into the second gel well. The electrophoresis tool is run with an electric current of 80 volts for 55 minutes. After the running process is complete, agarose gel is taken and observed on a UV transilluminator. While the measurement of DNA concentration and purity with a spectrophotometer and absorbance are adjusted at wavelengths (λ) 260 and 280 nm.

2.4 Sequencing with Next Generation Sequencing (NGS) Method and Data Analysis

This research uses the Illumina HiSeq Next Generation Sequencing (NGS) method. DNA samples that have been tested for results by visualizing and measuring the concentration and purity of the DNA are sent to Novogene, Singapore, for sequencing. OTU clustering analysis was performed using the Uparse software.

3. RESULTS AND DISCUSSION

Samples of cyprinus and tilapia were obtained from Cirata Reservoir and catfish samples were obtained from the Ciparanje FPIK Unpad Wet Laboratory. Administratively, Cirata Reservoir covers three districts in the West Java region, namely West Bandung, Purwakarta, and Cianjur Regencies. While the Ciparanje Wet Laboratory FPIK Unpad is located in Cileles, Jatinangor District, Sumedang Regency, West Java.

Table 1. Body Length, Weight, Gut Length and Stadia

No.	Sample	Body Length (cm)	Weight (gram)	Gut length (cm)	Stage
1.	Carp	23	240,34	27	Adult
2.	Tilapia	22	230	25	Adult
3.	Catfish	45	668,33	25	Adult

The results of length measurements in Table 1 found that the length of the gut in carp is longer than its body length. Tilapia have a gut length shorter than the body. Catfish have a shorter gut length than their bodies. By their nature, carp classified as omnivorous tend to be herbivorous [3, 11, 12] tilapia are classified as omnivores [13,14], and catfish that are classified as omnivorous fish tend to be carnivorous [4,15] have shorter bowel length than their body. This is in line with the fact that the herbivorous fish of the digestive tract several times its body length can reach five times its body length, while the intestinal length of carnivorous fish is shorter than the total body length and the intestinal length of omnivorous fish is only slightly longer than the total body [16].

The type of feed eaten is influenced by several factors namely certain types of feed, size, age of fish, season and habitat for life [17]. The type of feed to be eaten by fish depends on the availability of the type of feed in the feed and also the physiological adaptation of the fish such as intestinal length, the nature and physiological conditions of digestion, the shape of teeth and pharyngeal bones, body shape and behavior. While the amount of feed needed by fish depends on eating habits, the abundance of feed, the value of feed conversion and the condition of the fish feed [18].

The type of feed contained in the gut of fish affects the presence of bacteria in it. Also the presence of bacteria in the gut of fish is influenced by other factors such as fish species, fish age, environmental conditions, climate, and other stress factors. Bacteria in the gut among them have the main function to assist the metabolic process in converting feed into components that can be digested and absorbed by the body [19].

The samples obtained were then isolated by a procedure using the Quick-DNA ™ Fecal / Soil Microbe Miniprep Kit (Zymo Research, catalog no. D6010). The next step is to check the quality of the meta-genome DNA isolated using 1% agarose gel electrophoresis. The results of DNA electrophoresis obtained in Figure 1 showed that the DNA bands were isolated from samples of carp, tilapia, and catfish but there were smears. Based on the picture also seen the thickness of the band is a variety of this is caused by different DNA concentrations. Metagenome DNA has averaged over 10,000 bp (10 kb).

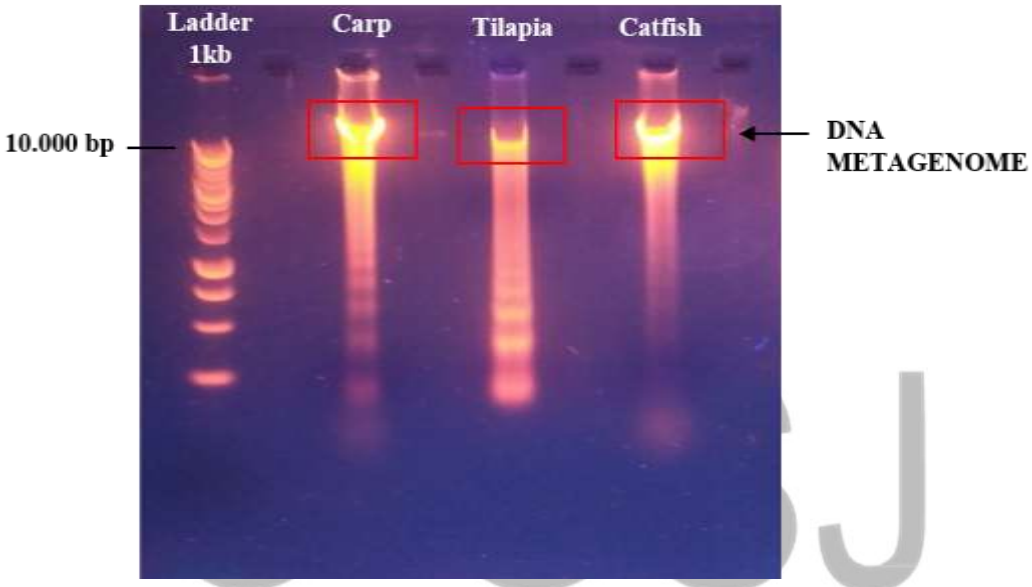

Figure 1. Agarose gel photo showing DNA metagenome bands from carp, tilapia, and catfish

The results obtained from the measurement of DNA purity and concentration through the calculation of the absorbance value of 260 nm divided by the absorbance value of 280 nm (A260 / A280) where the wavelength of UV light at 260 nm can be absorbed by DNA double fragments, while the wavelength of UV light at 280 nm can it is absorbed by protein or phenol contaminants so that by this measurement the level of purity in the genomic DNA can be known [20]. The results of the quantification of purity and concentration can be seen in Table 2.

Table 2. Purity and Concentration DNA Metagenome

No.	Sample	A260	A280	Purity (Ratio A260/A280)	Concentration (ng/µl)
1.	Carp	0,2943	0,1565	1,88	294,3
2.	Tilapia	0,0891	0,0459	1,94	89,1
3.	Catfish	0,0028	0,1049	1,92	201,8

DNA isolation results are pure if the A260 / A280 ratio is between 1.8 to 2.0 [21]. Ratio values lower than 1.8 indicate the presence of protein, salt or solvents, while ratio values above 2.0 indicate the presence of extracted RNA. Ratio values close to 2.90 indicate the presence of a small portion of RNA [22]. Based on Table 1 it was found that the ratio of purity of carp, tilapia, and catfish samples ranged from 1.88 to 1.94.

The concentration values in the three fish are different. The difference in DNA concentration obtained in each sample can be determined by the physical treatment given and the ability of the extraction buffer in breaking down cells. The process of cell destruction physically with perfect grinding can facilitate the extraction buffer in breaking down cells. Besides the extraction buffer used can affect the concentration of DNA produced [23].

The results of DNA concentration and purity measurements showed that the three samples had sufficient quality and quantity to be sequenced with 16S rRNA Next Generation Sequencing in Novogene, Singapore. The requirements for sequencing are minimum degradation, purity A260 / 280 is 1.8 - 2.0, concentrations > 50 ng / µL, and no gDNA and protein contamination [24]. These results indicate that all three samples are eligible to be continued at the next stage.

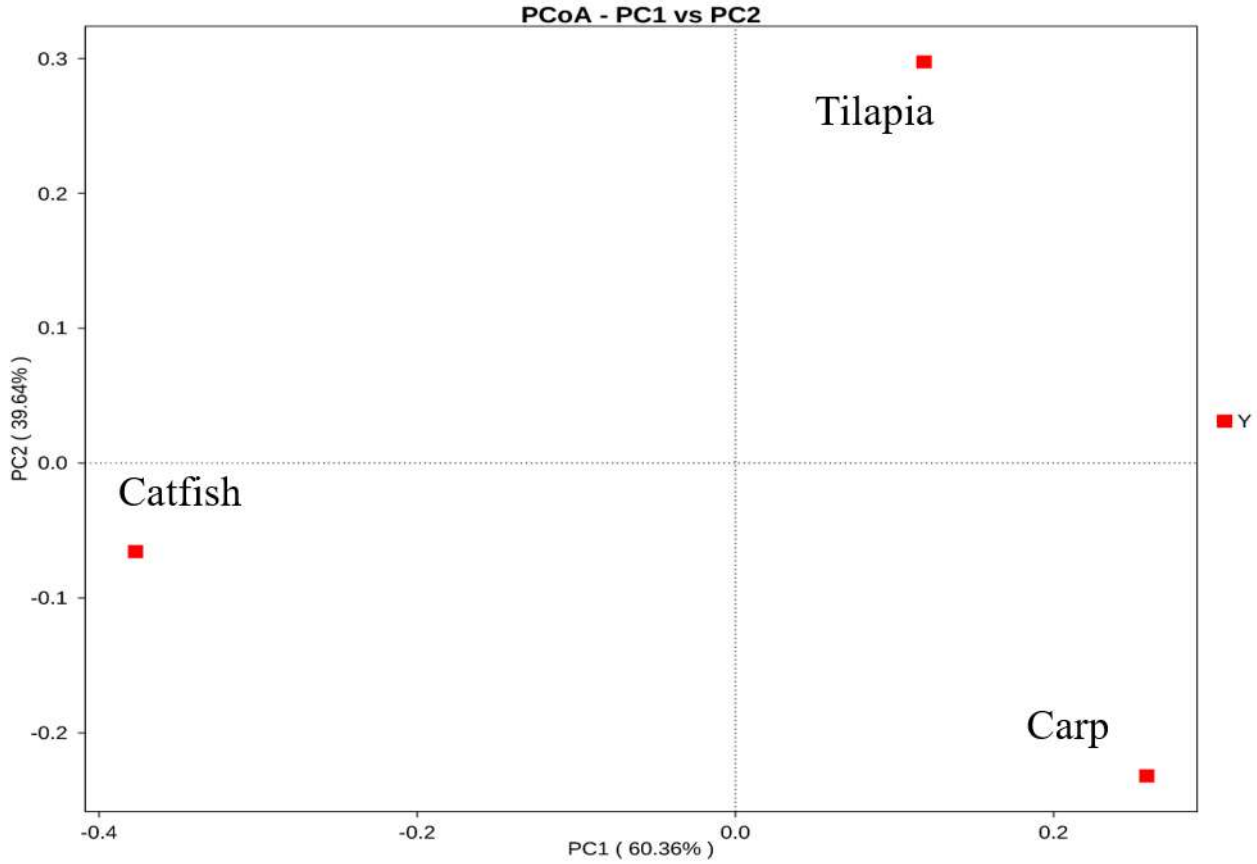

Figure 2. Principal coordinates analysis (PCoA) of bacterial community compositions in fish gut based on the unweighted UniFrac

The principal coordinates 1 (PC1) and 2 (PC2) values obtained from the graphs in Figure 2 are 60.36% and 39.64%, respectively. The grouping results found that the intestinal bacterial community of carp, tilapia, and catfish formed a different group. This is in line with the previous research that explains the PcoA scatter plot between eight fish samples from rivers that are omnivorous, herbivorous, carnivorous and filter feeders showing a clear separation of community composition [25].

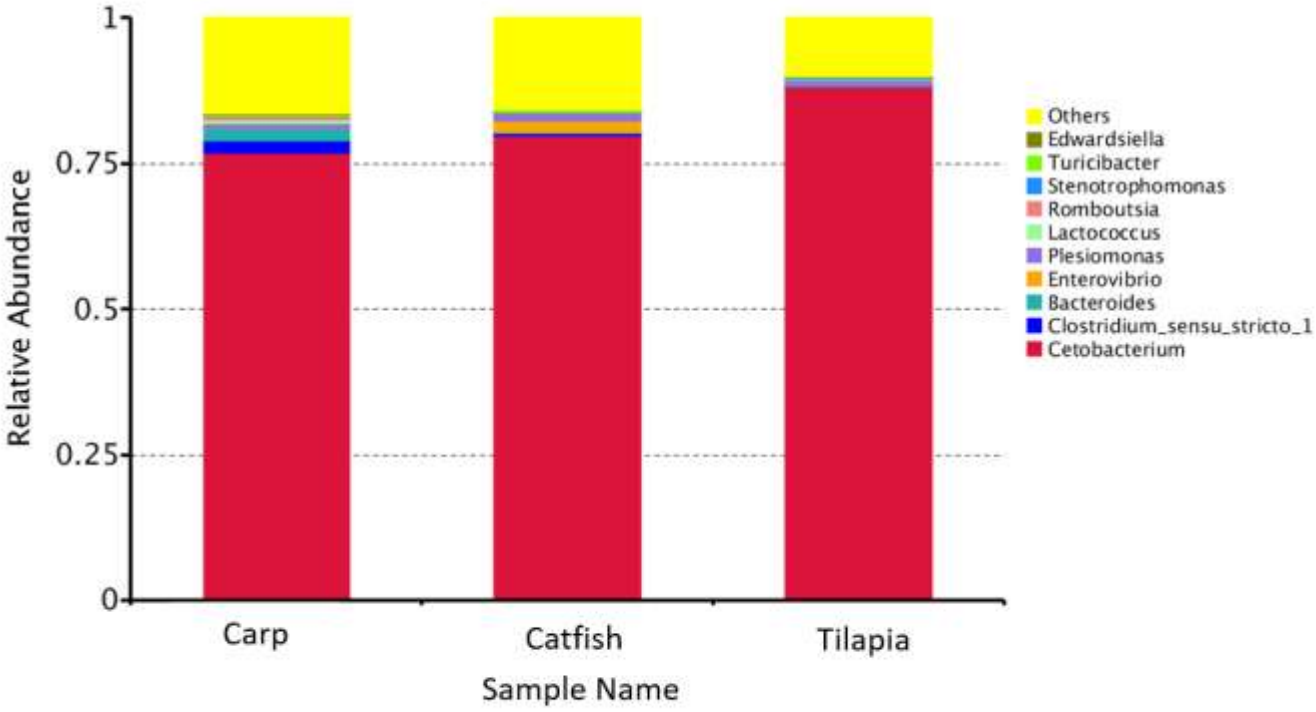

Figure 3. TOP10 Genus of Bacteria in Carp, Tilapia, and Catfish

Based on Figure 3, the highest abundance at the genus level is *Cetobacterium, Clostridium sensu stricto 1, Bacteroides, Enterovibrio, Plesiomonas, Lactococcus, Romboutsia, Stenotrophomonas, Turicibacter, Edwardsiella,* and Others. The abundance of the genus level is dominated by Cetobacterium, fish with relative abundance 0,76 in carp, 0,88 in tilapia, 0,79 in catfish. *Cetobacterium* is known to produce vitamin B12 and is found in plant feed in the gut [26,27,28].

The relative abundance *of Clostridium sensu stricto 1* was found in carp, tilapia, and catfish with relative abundace (0,02; 0,001;0,004). *Clostridium sensu stricto 1* is a genus representing *Clostridium cluster 1* in the 16S rRNA tree, this cluster is defined in phylogenetic terms, and no biochemical, molecular or phenotypic characteristics are known to be unique to the species of this cluster [29]. Clostridium sensu stricto 1 has similarities with *Clostridium thermocellum, Thermoanaerobacter pseudethanolicus, Thermoanaerobacter tengcogensi*s and *Caldicellulosiruptor saccharolyticus* [30].

Bacteria with the genus *Clostridium* are obligate gram-positive anaerobic bacteria with many pathogenic species. This bacterium has been shown to contribute to host nutrition, especially by supplying fatty acids and vitamins [31]. Other bacteria that predominate in all three fish are the genus *Bacteroides* with relative abundances 0,02 in carp, 0,001 in tilapia, and 0,0007 in catfish. This genus functions as carbohydrate fermentation which produces a collection of volatile fatty acids that are reabsorbed through the large gut and utilized by the host as an energy source, providing a significant proportion of the host's daily energy requirements [32]. *Bacteroides* have a function for fermenting carbohydrates which produce a collection of volatile fatty acids that are reabsorbed through the large gut and used as an energy source [33].

Bacteria that abundant only in carp and tilapia is *Enterovibrio* with the relative abundance 0,006 in carp and 0,009 in tilapia. The function of *Enterovibrio* is produced indole acetic acid which can be harmful to lactic acid bacteria in the gut if in excessive amounts [36,37]. Another genus that is high in all three fish is *Plesiomonas* with the relative abundances are 0,009 in carp and tilapia and 0,019 in catfish. Based on the results obtained

sequencing known that the species is P*lesiomonas shigelloides*. *Plesiomonas shigelloides* is a water bacterium and soil sediment that has the ability of proteolytic as well as including pathogenic bacteria which are detrimental to marine organisms [34]. This bacteria is also known to be pathogenic in Silver Carp [35]. Also there is *Lactococcus* the fifth-highest genus in carp and tilapia with an abundance of 0,0064 and 0,001. However, this genus is not found in catfish. This genus is known to be probiotic in tilapia [38].

The genus *Stenotrophomonas* is only identified in catfish, this genus functions as a cellulolytic species, associated with carboxymethyl cellulase (CMCase) or avicelase activity [39]. The genus *Turicibacter* serves to contain butyric acid, an important short-chain fatty acid with anti-microbial properties [40]. *Edwardsiella* is a pathogenic bacterium in aquaculture, more than 20 fish species are affected by this bacterial disease [38]. The high abundance of others at the genus level is due to the lack of databases and poor reads sequencing.

Based on previous research, it was found that *Clostridium, Citrobacter* and *Leptotrichia* are abundant bacteria in carnivorous fish, whereas in herbivorous fish the abundance in the genera Cetobacterium and Halomonas. In abundant bacteria omnivorous fish with the genus *Clostridium, Cetobacterium* and *Halomonas* [25]. Following the results obtained in carp, tilapia, and catfish where all three of these fish are omnivorous and bacterial abundance results obtained by Cetobacterium.

Conclusion

Intestinal bacterial communities of carp, tilapia, and catfish form different groups. The highest abundance of Cetobacterium, Clostridium sensu stricto 1, Bacteroides, Enterovibrio, Plesiomonas, Lactococcus, Romboutsia, Stenotrophomonas, Turicibacter, Edwardsiella, and Others.

References

[1] Effendi, Irzal. 2004. *Pengantar Akuakultur*. Jakarta: Penebar Swadaya.
[2] Ghanbari, M., W. Kneifel, and K. J. Domig. 2015. A new view of the fish gut microbiome: Advances from next-generation sequencing. *Aquaculture 448* 464-475.
[3] Li, j., J. Ni, J. Li, C. Wang, X. Li, S. Wu, T. Zhang, Y. Yu, and Q. Yan. 2014. "Comparative study on gastrointestinal microbiota of eight." *Journal of Applied Microbiology* ISSN 1364-5072
[4] Chen, T. P. 1976. *Aquaculture Practise in Taiwan.* Norwich: Page Bros.
[5] Bano, N., Smith, A.D., Bennett, W., Vasquez, L., and Hollibaugh, J.T. 2007. Dominance of Mycoplasma in the gut of the Long-Jawed Mudsucker, Gillichthys mirabilis, from five California salt marshes. *Environ Microbiol* 9, 2636–2641.
[6] Nayak, S.K. 2010 Probiotics and immunity: a fish perspective. *Fish Shellfish Immunol* 29, 2–14.
[7] Wu, S., Wang, G., Angert, E.R., Wang, W., Li, W. and Zou, H. 2013. Composition, Diversity, and origin of the bacterial community in grass carp gut. *PLoS ONE* 7,e3044
[8] Yan, Q., van der Gast, C.J. and Yu, Y. 2012. Bacterial community assembly and turnover within the gut of developing zebrafish. *PLoS ONE* 7, e30603.
[9] Ni J., Yan Q., Zhang T. 2013. Fish gut microecosystem: a model for detecting spatial pattern of microorganisms. *Chinese Journal of Oceanology and Limnology* Vol. 32 No. 1, P. 54-57
[10] Mulyani, Y. 2018. *Diversitas dan Peranan Bakteri Usus Ikan Mas Dalam Meningkatkan Sistem Imun dan Pengendalian Penyakit Ikan.* Bandung: Disertasi. Institut Teknologi Bandung.
[11] Susanto, H. 2007. *Budidaya Ikan di Pekarangan.* Jakarta : Penebar Swadaya.
[12] Naik, G., M. Rashid, MH. Balkhi, and FA. Bhat. 2015. Food and Feeding Habits of *Cyprinus carpio* var. communis: A Reason that Decline Schizothoracine Fish Production from Dal Lake of Kashmir Valley. *Fish and Aquaculture Journal Volume 6* 1-5.
[13] Khairuman, H., and K. Amri. 2013. *Budidaya Ikan Nila.* Jakarta: PT AgroMedia Pustaka.
[14] Vasconcelos, F. R., R. F. Menezes, and J. L. Attayde. 2018. Effects of the Nile tilapia (*Oreochromis niloticus L.*) on the plankton community of a tropical reservoir during and after an algal bloom. *Hydrobiologia* 1-9.
[15] Smith, L. S. 2015. *Chapter 1. Digestion in Teleost Fishes.* URL: http://www.fao.org/3/x5738e/x5738e02.htm. Accessed on 28 March 2019.
[16] Moyle P.B. and Jr. J.J. Cech. 2004. *Fishes.* An Introduction to Ichthyology. 5th. USA: Prentice Hall, Inc.
[17] Ariyanto, D. 2002. Analisis keragaman bentuk tubuh ikan nila strain gift pada tingkatan umur yang berbeda. *Jurnal Perikanan*, 4(1): 19-26.
[18] Antoni, A. 2012. *Biologi Ikan Mas* (C*yprinus carpio*). Laboratorium Kimia Fisik. Jurusan Kimia Fakultas ilmu Pengetahuan Alam. Universitas Diponegoro, Semarang
[19] Zoetendal, E. G., Collier, C. T., Koike, S., Mackie, R. I., & Gaskins, H. R. 2004. Molecular Ecological Analysis of the Gastrointestinal Microbiota: A Review. *The Journal of Nutrition*, 134(2), 465–472.
[20] Fatchiyah, E. L. Arumingtyas, S. Widiarti, and S. Rahayu. 2011. *Biologi Molekular Prinsip Dasar Analisis.* Jakarta: Penerbit Erlangga.
[21] Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual, 2nd ed*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
[22] Tan, Siun and Yiap, Beow Chin. 2009. DNA, RNA, and protein extraction: The past and the present. *Journal of biomedicine & biotechnology*. 574398.
[23] Mulyani, Y., A. Purwanto, and I. Nurruhwati. 2011. Perbandingan Beberapa Metode Isolasi DNA untuk Deteksi Dini Koi Herves Virus (KHV) pada Ikan Mas (*Cyprinus carpio L.*). *Jurnal Akuatika.* (2):1-16
[24] Novogene. 2018. *Metagemonic Sequencing.* URL: https://en.novogene.com/next-generation-sequencing-services/microbial-genome/metagenomic-sequencing/. Accessed on 16 Maret 2019.

[25] Liu, H., X. Guo, R. Lai, R. Gooneratne, R. Lai, C. Zeng, F. Zhan, and W. Wang. 2016. The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Scientific Reports* 6:23430 1-12.

[26] Sugita, H., Shibuya, K., Shimooka, H. and Deguchi, Y. 1996. Antibacterial abilities of intestinal bacteria in freshwater cultured fish. *Aquaculture 145*, 195–203.

[27] Wu, S., Wang, G., Angert, E.R., Wang, W., Li, W. and Zou, H. 2013 Composition, Diversity, and origin of the bacterial community in grass carp gut. *PLoS ONE* 7,e30440.

[28] Tsuchiya, C., Sakata, T., Sugita, H. 2008. Novel ecological niche of *Cetobacterium somerae*, an anaerobic bacterium in the intestinal tracts of freshwater fish. *Letters in Applied Microbiology* 46, 43–48.

[29] Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., FernandezGarayzabal, J., Garcia, P., Cai, J., Hippe, H., Farrow, J. A. E. & other authors. 1994. The phylogeny of the genus Clostridium: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* 44, 812–826.

[30] Gupta, Radhey & Gao, Beile. 2009. Phylogenomic analyses of clostridia and identification of novel protein signatures that are specific to the genus *Clostridium sensu stricto* (cluster I). I*nternational journal of systematic and evolutionary microbiology*. 59. 285-94.

[31] Balcazar, J., Blas, I., Ruizzarzuela, I., Cunningham, D., Vendrell, D., & Muzquiz, J. 2006. The role of probiotics in aquaculture. *Veterinary Microbiology,* 114(3-4), 173–186.

[32] Wexler, H. M. 2007. Bacteroides: the Good, the Bad, and the Nitty-Gritty. *Clin Microbiol Rev.* 20(4): 593–621.

[33] Göker M, Gronow S, Zeytun A, Nolan M, Lucas S, Lapidus A, Hammon N, Deshpande S, Cheng JF, Pitluck S, Liolios K, Pagani I, Ivanova N, Mavromatis K, Ovchinikova G, Pati A, Tapia R, Han C, Goodwin L, Chen A, Palaniappan K, Land M, Hauser L, Jeffries CD, Brambilla EM, Rohde M, Detter JC, Woyke T, Bristow J, Markowitz V, Hugenholtz P, Eisen JA, Kyrpides NC, Klenk HP. 2011. Complete genome sequence of Odoribacter splanchnicus type strain. *Stand Genomic Sci*. 4(2):200-9

[34] Pereira, S. C., Amorim, S. D., Santos, A. F. M., & Rodrigues, D. P. 2008. *Plesiomonas Shigelloides* And Aeromonadaceae Family Pathogens Isolated From Marine Mammals Of Southern And Southeastern Brazilian Coast. Universidade Federal do Rio Grande do Sul, Imbé, RS, Brasil. *Brazilian Journal of Microbiology*, 39:749-755.

[35] Bahera, B. K., Bera, A. K., Paria, P., Das A., Parida P.K., Suman Kumari, Bhowmick S., Das B.K. 2018. Identification and phatogenicity of Plesiomonas shigeloides in Silver Carp. Aquaculture (493); 314-318

[36] Hoeppli R. E., Wu D., Cook L., Levings M. K. 2015. The environment of regulatory T cell biology: cytokines, metabolites, and the microbiome. *Frontiers in Immunology*; 6, article no. 61

[37] Nowak A, Libudzisz Z. 2006. Influence of phenol, p-cresol and indole on growth and survival of intestinal lactic acid bacteria. *Anaerobe* 12:80–84

[38] Xia, Y., Cao, J., Wang, M., Lu, M., Chen, G., Gao, F., Yi, M. 2018. Effects of *Lactococcus lactis* subsp. lactis JCM5805 on colonization dynamics of gut microbiota and regulation of immunity in early ontogenetic stages of tilapia. *Fish & Shellfish Immunology.* 86:53-63

[39] Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, et al. 2012. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 490:55–60.

[40] Leal CAG, Carvalho-Castro GA, Sacchetin PSC, Lopes CO, Moraes AM, Figueiredo HCP. 2010. Oral and parenteral vaccines against *Flavobacterium columnare*: evaluation of humoral immune response by ELISA and in vivo efficiency in Nile tilapia (*Oreochromis niloticus)*. *Aquaculture International* 2;18:657–666.