STRUKTUR KOMUNITAS DAN KEANEKARAGAMAN BAKTERI PADA SALURAN PENCERNAAN SIDAT *ELVER* BUDIDAYA BERDASARKAN ANALISIS METAGENOMIK

SKRIPSI

diajukan untuk memenuhi sebagian syarat untuk memperoleh gelar Sarjana Sains
Program Studi Biologi Departemen Pendidikan Biologi

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UNIVERSITAS PENDIDIKAN INDONESIA
2020

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Universitas Pendidikan Indonesia

2020

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ABSTRAK

Ikan sidat (*Anguilla* sp.) merupakan ikan konsumsi yang sangat bernilai ekonomis baik di pasar lokal maupun luar negeri. Kandungan vitamin dan mikronutrien pada ikan sidat sangat tinggi. Hal ini menjadi faktor pendorong banyaknya pembudidayaan ikan sidat. Dibalik tingginya minat pasar, terdapat kendala dalam pembudidayaan yang berkaitan dengan keberlangsungan hidup ikan sidat, terutama pada fase *elver*. Salah satu aspek yang bekontribusi terhadap kesehatan ikan sidat adalah keseimbangan mikrobiota yang menghuni saluran pencernaan. Penelitian ini bertujuan untuk mempelajari struktur komunitas, keanekaragaman, dan kelimpahan bakteri yang menghuni saluran pencernaan ikan sidat *elver*. Metode yang digunakan untuk menyelidiki informasi terkait dengan komunitas bakteri adalah dengan cara mengambil sampel DNA genom bakteri total dari saluran pencernaan sidat *elver*. Konsentrasi dan kemurnian DNA dianalisis dengan ketentuan rasio OD 260/280. Analisis terhadap sampel dilakukan dengan metode *Next Generation Sequencing* dengan menargetkan daerah V3-V4 dari gen 16S rRNA. Analisis data dilakukan menggunakan perangkat lunak Mothur dan PAST v.3.26 untuk menghitung keanekaragaman. Semua fragmen 16S rRNA yang dihasilkan kemudian diklasifikasikan menjadi 19 filum, 73 ordo, 36 kelas, 124 famili, dan 210 genus. Proteobacteria (64%) ditemukan sebagai filum dengan kelimpahan tertinggi yang diikuti oleh Firmicutes (29%), dan filum lainnya. Mikrobiota saluran pencernaan sidat *elver* pada tingkat genus didominasi oleh bakteri *Plesiomonas*. Informasi yang diperoleh dari mikrobiota saluran pencernaan dapat berguna untuk menentukan kesehatan dan meningkatkan kondisi pemeliharaan dalam pembudidayaan ikan sidat.

Kata Kunci: Ikan sidat, *Anguilla* sp., Mikrobiota, Metagenomik, 16S rRNA, *Next Generation Sequencing*.

COMMUNITY STRUCTURE AND BACTERIAL DIVERSITY IN DIGESTIVE TRACT OF CULTIVATED *ELVER* EEL BASED ON METAGENOMIC ANALYSIS

ABSTRACT

Eel (*Anguilla sp*.) is a consumption fish that is very economical in both local and foreign markets.The content of vitamins and micronutrients in eel is very high. This matter become a driving factor for the number of eel fish farming. Behind the high market interest, there are obstacles in the cultivation of eels, especially in the *elver* phase. One aspect that contributes to the health of eel is the balance of the microbiota that inhabit the digestive tract. This research aims to study the community structure, diversity and abundance of bacteria that inhabit the digestive tract of *elver* eels. The method used to investigate information related to the bacterial community is by taking total bacterial genomic DNA from digestive tract of elver eel. The concentration and purity of the DNA obtained were analyzed using the OD ratio of 260/280. Examination of the samples was carried out using the Next Generation Sequencing method by targeting the V3-V4 regions of the 16S rRNA gene. Data analysis was performed using Mothur software and PAST v.3.26 for calculating alpha diversity. All 16S rRNA fragments produced were then classified into 19 phyla, 73 orders, 36 classis, 124 families, and 210 genera. Proteobacteria (64%) were found as the highest phylum followed by Firmicutes (29%), and other phyla. Digestive tract microbiota of *elver* eels was dominated by the genus *Plesiomonas*. Information obtained from the microbiota of the digestive tract can be useful for determining health and improving maintenance conditions in eel farming.

Keywords: Eel, *Anguilla sp*., Microbiota, Metagenomic, 16S rRNA, Next Generation Sequencing.

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DAFTAR PUSTAKA

Aarestrup, K., Okland, F., Hansen, M. M., Righton, D., Gargan, P., Castonguay, M., … McKinley, R. S. (2009). Oceanic Spawning Migration of the European Eel *(Anguilla anguilla). Science*, *325*(5948), 1660–1660. doi:10.1126/science.1178120 .

Affandi, R. (2005). Strategi pemanfaatan sumber daya ikan sidat. *Jurnal Iktiologi Indonesia*. 5(2):77-81.

Affandi, R., & Suhenda, N. (2003). “Teknik Budidaya Ikan Sidat (*Anguilla bicolor bicolor*)”. *Prosiding Sumberdaya Perikanan Sidat Tropik* (hal. 47 – 54). UPT Baruna Jaya – BPPT.

Affandi, R., Budiardi, T., Wahju, R. I., & Taurusman, A.A. (2013). Pemeliharaan Ikan Sidat dengan Sistem Air Bersirkulasi. *Jurnal Ilmu Pertanian Indonesia*, *18* (1), 55–60.

Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., & Narasimhan, G. (2016). Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis. *Evolutionary Bioinformatics, 12s1, EBO.S36436.* doi:10.4137/ebo.s36436.

Allimetrics.net. (2019). *DNA sequence analysis* [*Online*]. Diakses dari : http://www.alimetrics.net/en/index.php/dna-sequence-analysis.

Angreni, N.P.W., Arthana, I.W., & Suryaningtyas, E.W. (2018). Identifikasi Bakteri Patogen Pada Ikan Nila (*Oreochromis niloticus*) di Danau Batur, Bali. *Current Trends in Aquatic Science, 1*(1), 98-105.

Ansorge, W. J. (2009). Next-generation DNA sequencing techniques. *New Biotechnology*, *25*(4), 195–203. doi:10.1016/j.nbt.2008.12.009 .

Astuti, T. I. (2018). *Uji Meat Bone Meal (Mbm) Dan Poltry By- Product (Pbm) Pada Pakan Terhadap Laju Pertumbuhan Dan Sintasan Ikan Sidat (Anguilla Bicolor) Stadia Elver* . (Skripsi). Fakultas Pertanian Peternakan, Universitas Muhammadiyah Malang.

Asy’ari, M. & Noer, A. S. (2005). Optimasi Konsentrasi MgCl2 dan Suhu Annealing pada Proses Amplifikasi Multifragmens mtDNA dengan Metoda PCR. *Jurnal Kimia Sains dan Aplikasi,* *8*(1). doi:10.14710/jksa.8.1.23-27.

Bae, J., Kim, D., Yoo, K., Kim, S., Lee, J., & Bai, S. C. (2010). Effects of dietary arachidonic acid (20:4n-6) levels on growth performance and fatty acid composition of juvenile eel *Anguilla japonica. Asian Australasian Journal of Animal Sciences*, *23*(4). doi:10.5713/ajas.2010.90491.

Bai, L., Sun, H., Zhang, X., & Cai, B. (2018). Next-generation sequencing of root fungal communities in continuous cropping soybean. *Chilean Journal of Agricultural Research, 78*(4), 528–538. doi:10.4067/s0718 58392018000400528.

Balcázar, J. L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I., Muzquiz, J. L., & Girones, O. (2008). Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. *Aquaculture*, *278*(1-4), 188–191.

Barcenilla, A., Pryde, S.E., Martin, J.C., Duncan, S.H., Stewart, C.S., Henderson C., & Flint, H.J. (2000). Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol*, *66*,1654–1661. doi:10.1128/aem.66.4.1654-1661.2000.

Belstrøm, D., Constancias, F., Liu, Y., Yang, L., Drautz-Moses, D. I., Schuster, S. C., … Givskov, M. (2017). Metagenomic and metatranscriptomic analysis of saliva reveals disease-associated microbiota in patients with periodontitis and dental caries. *Npj Biofilms and Microbiomes*, *3*(1). doi:10.1038/s41522-017-0031-4.

Benucci, G. M. N., Bonito, V., & Bonito, G. (2018). Fungal, Bacterial, and Archaeal Diversity in Soils Beneath Native and Introduced Plants in Fiji, South Pacific. *Microbial Ecology, 78*(3) doi:10.1007/s00248-018-1266-1.

Bergmann, G. T., Craine, J. M., Robeson, M. S., & Fierer, N. (2015). Seasonal Shifts in Diet and Gut Microbiota of the American Bison (*Bison bison). PLOS ONE,* 10(11):e0142409. doi:10.1371/journal.pone.0142409.

Bhat, A. H., & Prabhu, P. (2017). OTU Clustering: A Window to Analyse Uncultured Microbial World. *International Journal of Scientific Research in Computer Science and Engineering,* *5*(6), 62-68. doi: 10.26438/ijsrcse/v5i6.6268

Biddle, A., Stewart, L., Blanchard, J., & Leschine, S. (2013). Untangling the genetic basis of fibrolytic specialization by Lachnospiraceae and Ruminococcaceae in diverse gut communities. *Diversity*, *5*, 627–640. doi:10.3390/d5030627.

Buzas, M.A. & Gibson T.G. (1969). Species diversity: benthonic foraminifera in western North Atlantic. *Science*, *163*, 72-75.

Cahill, M.M. (1990). Bacterial flora of the fishes. *Microb Ecol.*, *19*, 21-41. doi: 10.1007/BF02015051.

Cai, H, Archambault, M., Prescott, J.F. (2003). 16S Ribosomal RNA Sequence–based Identification of Veterinary Clinical Bacteria. *J Vet Diagn Invest.*, *15*, 465–469. doi:10.1177/104063870301500511.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335–336. doi:10.1038/nmeth.f.303.

Carding, S., Verbeke, K., Vipond, D. T., Corfe, B. M., & Owen, L. J. (2015). Dysbiosis of the gut microbiota in disease. *Microbial Ecology in Health & Disease, 26*(0), 26191. doi:10.3402/mehd.v26.26191.

Carrasco, J. M. D., Casanova, N.A., Miyakawa, M. E. F. (2019). Microbiota, Gut Health and Chicken Productivity: What Is the Connection?. *Microorganisms, 7*, 374. doi10.3390/microorganisms7100374.

Chakravorty, S., Helb, D., Burday, M., Connell, N., & Alland, D. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods, 69*(2), 330–339. https://doi.org/10.1016/j.mimet.2007.02.005.

Chamberlain, L. A., Bolton, M. L., Cox, M. S., Suen, G., Conley, S. P., & Ané, J.-M. (2020). Crop rotation, but not cover crops, influenced soil bacterial community composition in a corn-soybean system in southern Wisconsin. *Applied Soil Ecology, 154*, 103603. doi:10.1016/j.apsoil.2020.103603.

Chen, H., Li, C., Liu, T., Chen, S., & Xiao, H. (2019). A Metagenomic Study of Intestinal Microbial Diversity in Relation to Feeding Habits of Surface and Cave-Dwelling *Sinocyclocheilus* Species. *Microbial Ecology, 79,* 299–311. doi:10.1007/s00248-019-01409-4.

Chistoserdova, L. (2009).Functional Metagenomics: Recent Advances and Future Challenges. *Biotechnology & genetic engineering reviews, 26*(1),335-345. doi: 10.5661/bger-26-335

Claesson, M. J., Wang, Q., O’Sullivan, O., Greene-Diniz, R., Cole, J. R., Ross, R. P., & O’Toole, P. W. (2010). Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Research, 38*(22), e200–e200. doi: 10.1093/nar/gkq873.

Clements, Frederic E., & Victor E. Shelford. (1939). *Bio-ecology*. New York: John Wiley & Sons.

Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., … Farrow, J. A. E. (1994). The Phylogeny Of The Marga *Clostridium*: Proposal Of Five New Genera And Eleven New Species Combinations. *International Journal Of Systematic Bacteriology, 44*(4), 812–826. Doi:10.1099/00207713-44-4-812.

Desai, A. R., Links, M. G., Collins, S. A., Mansfield, G. S., Drew, M. D., Van Kessel, A. G., & Hill, J. E. (2012). Effects of plant-based diets on the distal gut microbiome of rainbow trout *(Oncorhynchus mykiss). Aquaculture,* 350-353, 134–142. doi:10.1016/j.aquaculture.2012.04.005.

Dewi, N. & Koesharyani, I. (2017). Studies On *Aeromonas hydrophila* Bacteria Diseases in Wild and Cultured Elver Eel (*Anguilla bicolor*). *Indonesian Aquaculture Journal, 12* (2), 77-82.

Djurhuus, A., Pitz, K., Sawaya, N. A., Rojas-Márquez, J., Michaud, B., Montes, E., … Breitbart, M. (2018). Evaluation of marine zooplankton community structure through environmental DNA metabarcoding. *Limnology and Oceanography: Methods, 16*(4), 209–221. doi:10.1002/lom3.10237.

Dong, X., & Strous, M. (2019). An Integrated Pipeline for Annotation and Visualization of Metagenomic Contigs. *Frontiers in Genetics, 10*, 999. doi:10.3389/fgene.2019.00999.

Dulski, T., Zakęś, Z., & Ciesielski, S. (2017). Characterization of the gut microbiota in early life stages of pikeperch *Sander lucioperca* . *Journal of Fish Biology, 92*(1), 94–104. doi:10.1111/jfb.13496.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). *UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, 27*(16), 2194–2200. doi:10.1093/bioinformatics/btr381.

Eichmiller, J.J., Hamilton, M.J., Staley, C., Sadowsky, M.J. & Sorensen, P.W. (2016) Environment shapes the fecal microbiome of invasive carp species. *Microbiome, 4,* 44. doi: 10.1186/s40168-016-0190-1.

Esteve-Gassent, M. D., Nielsen, M. E., & Amaro, C. (2003). The kinetics of antibody production in mucus and serum of European eel (*Anguilla anguilla* L.) after vaccination against *Vibrio vulnificus*: development of a new method for antibody quantification in skin mucus. *Fish & Shellfish Immunology, 15*(1), 51–61. doi:10.1016/s1050-4648(02)00138-9.

Fahmi, M. R. (2015). “Short Communication: Conservation Genetic Of Tropical Eel In Indonesian Waters Based On Population Genetic Study”. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia* (hlm. 38-43). Depok: Balai Penelitian dan Pengembangan Budidaya Ikan Hias.

Farrell, R.E. (2005). *RNA methodologies: A laboratory guide for isolation and characterization.* (Edisi Ketiga). Burlington: Elsevier Academic Press.

Fastawa, Agustina, E., & Kamal, S. (2018). “Keanekaragaman Makrozoobenthos Sebagai Bioindikator Pencemaran di Kawasan Payau Krueng Aceh”. *Prosiding Seminar Nasional Biotik*, 5(1).

Feng, G., Xie, T., Wang, X., Bai, J., Tang, L., Zhao, H., … Zhao, Y. (2018). Metagenomic analysis of microbial community and function involved in cd-contaminated soil. *BMC Microbiology*, *18*(1). doi:10.1186/s12866-018-1152-5.

Flint, H. J., Scott, K. P., Louis, P., & Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology & Hepatology, 9*(10), 577–589. doi:10.1038/nrgastro.2012.156.

Food and Agriculture Organization. (2014). *Globefish Research Programme, Eel Anguilla spp.: Production and Trade*. Rome, Italia: FAO Fishstat Plus.

Fritsch, R. J., & Krause, I. (2003). ELECTROPHORESIS. *Encyclopedia of Food Sciences and Nutrition*, 2055–2062. doi:10.1016/b0-12-227055-x/01409-7.

Gajardo, K., Rodiles, A., Kortner, T. M., Krogdahl, Å., Bakke, A. M., Merrifield, D. L., & Sørum, H. (2016). A high-resolution map of the gut microbiota in Atlantic salmon (*Salmo salar*): A basis for comparative gut microbial research. *Scientific Reports*, *6*(1). doi: 10.1038/srep30893.

Goecks, J., Nekrutenko, A., & Taylor, J. (2010). Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol*., *11*(8),R86.doi: 10.1186/gb-2010-11-8-r86.

Gupta, R. S., & Gao, B. (2009). Phylogenomic analyses of clostridia and identification of novel protein signatures that are specific to the marga *Clostridium sensu stricto (cluster I). INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 59*(2), 285–294. doi:10.1099/ijs.0.001792-0.

Hajibabaei, M., Shokralla, S., Zhou, X., Singer, G.A.C, Baird, D.J. (2011) Environmental barcoding: a next-generation sequencing approach for biomonitoring applications using river benthos. *PLoS ONE,* 6(4):e1749. doi: 10.1371/journal.pone.0017497

Hamady, M., Walker, J.J., Harris, J.K., Gold, N.J., & Knight, R., (2008). Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nat. Methods, 5* (3), 235–237. doi:10.1038/nmeth.1184.

Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J., & Goodman, R. M. (1998). Molecular biological access to the chemistry of unknown soil microbes: A new frontier for natural products. *Chemistry and Biology*, *5*(10). doi: 10.1016/S1074-5521(98)90108-9.

Hanzen, C. (2017). *Anguilla bicolor bicolor* {subspecies} [*Online*]. Diakses dari : http://v3.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxid=462487 [12 November 2019].

Helvoort, B.V. (1981). *Bird Populations in The Rural Ecosystems of West Java*.. Netherlands: Nature Conservation Department

Henkel, C. V., Burgerhout, E., de Wijze, D. L., Dirks, R. P., Minegishi, Y., Jansen, H. J., … van den Thillart, G. E. E. J. M. (2012). Primitive Duplicate Hox Clusters in the European Eel's Genome. *PLoS ONE,* 7(2): e32231. doi:10.1371/journal.pone.003223.

Hennersdorf, P., Kleinertz, S., Theisen, S., Abdul-Aziz, M., Mrotzek, G., Palm, H.W. and Saluz, H.P. (2016) Microbial diversity and parasitic load in tropical fish of different environmental conditions. *PLoS ONE,* 11(3): e0151594.

Hong, C., Manimaran, S., Shen, Y., Perez-Rogers, J. F., Byrd, A. L., Castro-Nallar, E., … Johnson, W. (2014). PathoScope 2.0: a complete computational framework for strain identification in environmental or clinical sequencing samples. *Microbiome, 2*(1), 33. doi:10.1186/2049-2618-2-33.

Hsu, H.-Y., Chang, F.-C., Wang, Y.-B., Chen, S.-H., Lin, Y.-P., Lin, C.-Y., & Han, Y.-S. (2018). Revealing the compositions of the intestinal microbiota of three Anguillid eel species using 16S rDNA sequencing. *Aquaculture Research, 49*(7), 2404–2415. doi:10.1111/are.13700.

Huang, W., Cheng, Z., Lei, S., Liu, L., Lv, X., Chen, L., … Song, Y. (2018). Community composition, diversity, and metabolism of intestinal microbiota in cultivated European eel (*Anguilla anguilla*). *Applied Microbiology and Biotechnology, 102*(9), 4143–4157. https://doi.org/10.1007/s00253-018-8885-9.

Huang, Z., Li, X., Wang, L., & Shao, Z. (2014). Changes in the intestinal bacterial community during the growth of white shrimp, *Litopenaeus vannamei*. *Aquaculture Research*, *47*(6), 1737–1746. doi:10.1111/are.12628.

Hughes, J.B., Hellmann, J.J., Ricketts, T.H., Bohannan, B.J. (2001). Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl. Environ. Microbiol*. *67*, 4399-4406.

Husnah., Tjahjo, D. W. H., Nastiti, A., Oktaviani, D., Nasution, S. H., Sulistiono. (2008). *Status Keanekaragaman Hayati Sumberdaya Perikanan Perairan umum di Sulawesi*. Balai Riset Perikanan Perairan Umum, Pusat Riset Perikanan Tangkap. Badan Riset Kelautan dan Perikanan.

Igbinosa, I.H., Igumbor, E.U., Aghdasi, F., Tom, M., & Okoh, A.I., (2012). Emerging *Aeromonas* species infections and their significance in public health. *Sci. World J.*, 625023. doi: 10.1100/2012/625023.

Ingerslev, H. C., Strube, M. L., Jørgensen, L. V., Dalsgaard, I., Boye, M., & Madsen, L. (2014). Diet type dictates the gut microbiota and the immune response against Yersinia ruckeri in rainbow trout (*Oncorhynchus* *mykiss*). *Fish & Shellfish Immunology, 40*(2), 624–633. https://doi.org/10.1016/j.fsi.2014.08.021.

Jami, M., Ghanbari, M., Kneifel, W., Domig, K.J. (2015). Phylogenetic diversity and biological activity of culturable Actinobacteria isolated from freshwater fish gut microbiota. *Microbiol Res, 175,* 6-15.doi:10.1016/j.micres.2015.01.009.

Jiang, H., Dong, H., Zhang, G., Yu, B., Chapman, L. R., & Fields, M. W. (2006). Microbial Diversity in Water and Sediment of Lake Chaka, an Athalassohaline Lake in Northwestern China. *Applied and Environmental Microbiology*, *72*(6), 3832–3845. doi:10.1128/aem.02869-05.

Jiji Press. (2020). *Glass Eel Poaching on Rise in Japan amid Surging Prices* [*Online*]. Diakses dari : https://www.nippon.com/en/news/yjj2020012700688/glass-eel-poaching-on-rise-in-japan-amid-surging-prices.html.

Joh, S.-J., Ahn, E.-H., Lee, H.-J., Shin, G.-W., Kwon, J.-H., & Park, C.-G. (2013). Bacterial pathogens and flora isolated from farm-cultured eels *(Anguilla japonica)* and their environmental waters in Korean eel farms. *Veterinary Microbiology, 163*(1-2), 190–195. doi:10.1016/j.vetmic.2012.11.004

Kadri, K. (2020). Polymerase Chain Reaction (PCR): Principle and Applications. *Synthetic Biology - New Interdisciplinary Science*. doi:10.5772/intechopen.86491.

Kaper, J.B., Nataro, J.P., & Mobley, H.L. (2004). Pathogenic *Escherichia coli*. *Nat Rev Microbiol, 2*(2): 123-140. doi: 10.1038/nrmicro818.

Kementerian Kelautan dan Perikanan. (2015). *Rencana Aksi Nasiona Konservasi Sidat Period I: 2016-2020.* Jakarta: Direktorat Konservasi dan Keanekaragaman Hayati Laut.

Kohl, K. D., Oakeson, K. F., Orr, T. J., Miller, A. W., Forbey, J. S., Phillips, C. D., … Dearing, M. D. (2018). Metagenomic sequencing provides insights into microbial detoxification in the guts of small mammalian herbivores (*Neotoma* spp.). *FEMS Microbiology Ecology, 94*(12). doi:10.1093/femsec/fiy184.

Koontz, L. (2013). Agarose Gel Electrophoresis. *Laboratory Methods in Enzymology: DNA, 35–45.* doi:10.1016/b978-0-12-418687-3.00004-5 .

Kordi, M.G.H. (2004). *Penanggulangan Hama dan Penyakit Ikan*. Jakarta: Rineka Cipta.

Kowalczyk, R., Taberlet, P., Coissac, E., Valentini, A., Miquel, C., Kamiński, T., & Wójcik, J. M. (2011). Influence of management practices on large herbivore diet—Case of European bison in Białowieża Primeval Forest

(Poland). *Forest Ecology and Management*, *261*(4), 821–828. doi:10.1016/j.foreco.2010.11.026

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied and Environmental Microbiology, 79*(17), 5112–5120. doi:10.1128/aem.01043-13.

Kozińska, A., Paździor, E., Pękala, A., & Niemczuk, W. (2014). Acinetobacter johnsonii and Acinetobacter lwoffii - the emerging fish pathogens. *Bulletin of the Veterinary Institute in Pulawy, 58*(2), 193–199. doi:10.2478/bvip-2014-0029.

Krebs, C.J. (1978). *Ecological Methodology*. New York: Harper dan Row Publisher.

Kusen, K.O., Tumbol, R.A., & Manoppo, I. (2015). Identifikasi Penyakit Bakterial Pada Benih Sidat (*Anguilla marmorata*) di Balai Budidaya Air Tawar Tatelu. *Jurnal Budidaya Perairan Januari, 3*(1), 68-73.

Kusumawaty, D., Surtikanti, H. K., Hernawati, & Tallei, T. E. (2020). Data on community structure and diversity of the intestinal bacteria in elver and fingerling stages of wild Indonesian shortfin eel (*Anguilla bicolor bicolor*). *Data in Brief,* 29, 105299. doi:10.1016/j.dib.2020.105299.

Laitinen, K., & Mokkala, K. (2019). Overall Dietary Quality Relates to Gut Microbiota Diversity and Abundance. *International Journal of Molecular Sciences, 20*(8), 1835. doi:10.3390/ijms20081835.

Lakshminarayanan, B., Harris, H. M. B., Coakley, M., O’Sullivan, O., Stanton, C., … Pruteanu, M. (2012). Prevalence and characterization of *Clostridium perfringens* from the faecal microbiota of elderly Irish subjects. *Journal of Medical Microbiology*, *62*(Pt_3), 457–466. doi:10.1099/jmm.0.052258-0.

Lamb, E. G., Winsley, T., Piper, C. L., Freidrich, S. A., & Siciliano, S. D. (2016). A high-throughput belowground plant diversity assay using next-generation sequencing of the trnL intron. *Plant and Soil*, *404*(1-2), 361–372. doi:10.1007/s11104-016-2852-y.

Lan, V. T. T., Loan, P. T. T., Duong, P. A. T., Thanh, L. T., Ha, N. T., & Thuan, T. B. (2012). Straightforward Procedure for Laboratory Production of DNA Ladder. *Journal of Nucleic Acids, 1–4.* doi:10.1155/2012/254630 .

Lara, L.J. & Rostagno, M.H. (2013) . Impact of Heat Stress on Poultry Production. *Animals, 3,* 356–369. doi: 10.3390/ani3020356.

Larsen, A.M., Mohammed, H.H. & Arias, C.R. (2014) Characterization of the gut microbiota of three commercially valuable warmwater fish species. *J Appl Microbiol, 116,* 1396–1404. doi: 10.1111/jam.12475.

Lategan, M. J., F. R Torpy, & L. F. Gibson. (2004). Control of saprolegniosis in the eel *Anguilla australis* Richardson, by *Aeromonas media* strain A199. *Aquaculture, 240,* 19-27. doi: 10.1016/j.aquaculture.2004.04.009.

Lau, S.K.P., Woo, P.C.Y., Teng, J.L.L., Leung, K.W., & Yuen, K.Y. (2002). Identification by 16S Ribosomal RNA Gene Sequencing of *Arcobacter butzleri* Bacteraemia in a Patient with Acute Gangrenous Appendicitis. *J Clin Pathol*: *Mol Pathol., 55*(3), 182–185. doi: 10.1136/mp.55.3.182.

Lemos, L.N., Fulthorpe, R.R., Triplett, E.W., & Roesch, L.F. (2011). Rethinking microbial diversity analysis in the high throughput sequencing era. *J. Microbiol. Methods, 86*(1),42-51. doi: 10.1016/j.mimet.2011.03.014.

Lestari, N.W., Budiharjo, A., Pangastuti , A. (2016) . Bakteri heterotrof aerobik asal saluran pencernaan ikan sidat (*Anguilla bicolor bicolor*) dan potensinya sebagai probiotik. *Bioteknologi, 13* (1), 9-17. doi: 10.13057/biotek/c130102.

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences, 102*(31), 11070–11075. doi:10.1073/pnas.0504978102.

Li, J., Ni, J., Li, J., Wang, C., Li, X., Wu, S., … Yan, Q. (2014). Comparative study on gastrointestinal microbiota of eight fish species with different feeding habits. *Journal of Applied Microbiology, 117*(6), 1750–1760. doi:10.1111/jam.12663.

Liu, H., Guo, X., Gooneratne, R., Lai, R., Zeng, C., Zhan, F. and Wang, W. (2016). The gut microbiota and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. *Sci Rep.,* 6, 24340.

Liu, Z., DeSantis, T.Z., Andersen, G.L., Knight, R., (2008). Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic Acids Res., 36*(18), e120. https://doi.org/10.1093/nar/gkn491.

Llewellyn, M. S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G. R., … Derome, N. (2015). The biogeography of the atlantic salmon (Salmo salar) gut microbiome. *The ISME Journal, 10*(5), 1280–1284. doi:10.1038/ismej.2015.189.

Ludwig, W., Schleifer, K.-H., & Whitman, W. B. (2015). *Bacilli class. nov. Bergey's Manual of Systematics of Archaea and Bacteria,* 1–16. doi:10.1002/9781118960608.bm00025

Mahyarudin. (2014). *Studi metagenomik aktinomisetes berdasarkan gen 16s rRNA dan deteksi gen nifH pada tanah dan akar empat varietas tanaman padi dengan teknik DGGE*. (Tesis). Sekolah Pascasarjana, Institut Pertanian Bogor, Bogor.

Manurung, U.N. (2017). Identifikasi Bakteri Patogen Pada Ikan Nila (*Oreochromis niloticus)* di Lokasi Budidaya Ikan Air Tawar Kabupaten Kepulauan Sangihe. *Prosiding Seminar Nasional KSP2K II, 1*(2), 186 – 193.

Margalef, R., & Buzzati-Traverso, A. A. (1958). Perspectives in marine biology. In Temporal succession and spatial heterogeneity in phytoplankton (hlm. 323-347). Berkeley: University of California Press.

Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwälder, B., Schlötterer, C., & Boenigk, J. (2010). Diversity in a hidden world: potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Molecular Ecology, 19,* 32–40. doi:10.1111/j.1365-294x.2009.04478.x

Mekuchi, M., Asakura, T., Sakata, K., Yamaguchi, T., Teruya, K., & Kikuchi, J. (2018). Intestinal microbiota composition is altered according to nutritional biorhythms in the leopard coral grouper *(Plectropomus leopardus). PLOS ONE,* 13(6): e0197256. doi:10.1371/journal.pone.0197256.

Metch, J. W., Burrows, N. D., Murphy, C. J., Pruden, A., & Vikesland, P. J. (2018). Metagenomic analysis of microbial communities yields insight into impacts of nanoparticle design. *Nature Nanotechnology*, *13*(3), 253–259. doi:10.1038/s41565-017-0029-3.

Metzker, M. (2010). Sequencing technologies. The next generation. *Nat. Rev. Genet*. 11, 31–46. doi: 10.1038/nrg2626.

Mou, X., Sun, S., Edwards, R. A., Hodson, R. E., & Moran, M. A. (2008). Bacterial carbon processing by generalist species in the coastal ocean. *Nature, 451*(7179), 708–711. doi:10.1038/nature06513.

Mueller, K., Ash, C., Pennisi, E., & Smith, O. (2012). The Gut Microbiota. *Science, 336*(6086), 1245–1245. doi: 10.1126/science.336.6086.1245.

Murtini, Sri. (2010). *Makanan Alami Dan Perkembangan Anatomi Saluran Pencernaan Ikan Sidat (Anguilla Bicolor Bicolor Mcclelland 1844) Dari Muara Sungai Cimandiri Pelabuhan Ratu Jawa Barat*. (Tesis). Sekolah Pascasarjana, Institut Pertanian Bogor.

Nagamatsu, K., Hannan, T. J., Guest, R. L., Kostakioti, M., Hadjifrangiskou, M., Binkley, J., … Hultgren, S. J. (2015). Dysregulation of Escherichia coliαhemolysin expression alters the course of acute and persistent urinary tract infection. *Proceedings of the National Academy of Sciences, 112*(8), E871–E880. doi:10.1073/pnas.1500374112.

Nayak, S. K. (2010). Role of gastrointestinal microbiota in fish. *Aquaculture Research, 41*(11), 1553–1573. doi:10.1111/j.1365-2109.2010.02546.x

Nelson, J.S. (1994). *Fishes of the world*. (Third edition). New York: John Wiley & Sons, Inc.

Nento, R., Sahami, F., & Nursinar, S. (2013). Kelimpahan, Keanekaragaman dan Kemerataan Gastropoda di Ekosistem Mangrove Pulau Dudepo, Kecamatan Anggrek, Kabupaten Gorontalo Utara. *Nikè: Jurnal Ilmiah Perikanan dan Kelautan, 1*(1), 41-47.

Newman, M. M., Liles, M. R., & Feminella, J. W. (2015). Litter Breakdown and Microbial Succession on Two Submerged Leaf Species in a Small Forested Stream. *PLOS ONE*, 10(6):e0130801. doi:10.1371/journal.pone.0130801 .

Novogene. (2018). *Metagemonic Sequencing.* Diakses dari https://en.novogene.com/next-generation-sequencing-services/microbial-genome/metagenomic-sequencing/.

O’Neill, M., McPartlin, J., Arthure, K., Riedel, S., & McMillan, N. (2011). Comparison of the TLDA with the Nanodrop and the reference Qubit system. *Journal of Physics: Conference Series*, *307*, 012047. doi:10.1088/1742-6596/307/1/012047.

O'Callaghan, A., & van Sinderen, D. (2016). Bifidobacteria and Their Role as Members of the Human Gut Microbiota. *Frontiers in Microbiology 7*(925). doi: 10.3389/fmicb.2016.00925.

O’Connor, W. (2013). *VIDEO ON INCREASE IN SEVERN ELVER RUN 2013* [*Online*]. Diakses dari : https://europeaneel.com/2013/04/18/elevers/.[18 Agustus 2020].

Odum, E.P. (1993). *Dasar-dasar Ekologi*. (Edisi Ketiga). Yogyakarta: Gadjah Mada University Press.

Olson, N. D., Treangen, T. J., Hill, C. M., Cepeda-Espinoza, V., Ghurye, J., Koren, S., & Pop, M. (2017). Metagenomic assembly through the lens of validation: recent advances in assessing and improving the quality of genomes assembled from metagenomes. *Briefings in Bioinformatics, 20*(4),1140-1150. doi:10.1093/bib/bbx098.

Omenwa, V.C., Mbakwem-Aniebo, C., & Ibiene, A.A. (2015). Effects of selected probiotics on the growth and survival of fry–fingerlings of *Clarias gariepinus*. *Journal of Pharmacy and Biological Sciences, 10,* 89-93. doi: 10.9790/3008-10518993

Osimani, A., Milanović, V., Roncolini, A., Riolo, P., Ruschioni, S., Isidoro, N., … Clementi, F. (2019). *Hermetia illucens* in diets for zebrafish (Danio rerio): A study of bacterial diversity by using PCR-DGGE and metagenomic sequencing. *PLOS ONE*, 14(12): e0225956. doi:10.1371/journal.pone.0225956.

Pakingking, R. Jr, Palma, P., & Usero, R. (2015). Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines. *World J Microbiol Biotechnol, 31*(2),265–275. http://dx.doi.org/10.1007/s11274-014-1758-1.

Pandit, R. J., Hinsu, A. T., Patel, N. V., Koringa, P. G., Jakhesara, S. J., Thakkar, J. R., … Joshi, C. G. (2018). Microbial diversity and community composition of caecal microbiota in commercial and indigenous Indian chickens determined using 16s rDNA amplicon sequencing. *Microbiome, 6(1)*. doi:10.1186/s40168-018-0501-9.

Patantis, G., Januar, H.I., & Chasanah, E. (2012). Korelasi Keanekaragaman Bakteri terhadap Metabolit Sekunder *Nephthea* spp. dari Perairan Taman Nasional Kepulauan Seribu. *Widyariset*, *15*(3), 509-518.

Pielou, E.C. (1966). The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, *13,* 131–144. doi:10.1016/0022-5193(66)90013-0.

Pinoke, S.A.J., Tumbol, R.A. & Kolopita, M.E.F. (2015). Penambahan bakasang pada pakan benih sidat (*Anguilla marmorata*) untuk meningkatkan sistem imun non spesifik. *Budidaya Perairan Septemberi, 3*(3), 12- 18.

Poolsawat, L., Li, X., He, M., Ji, D., & Leng, X. (2019). *Clostridium butyricum* as probiotic for promoting growth performance, feed utilization, gut health and microbiota community of tilapia (*Oreochromis niloticus × O. aureus). Aquaculture Nutrition.*.doi:10.1111/anu.13025.

Protocol GoTaq Green Master Mix (2016). GoTaq® Green Master Mix (M712) Protocol. Diakses dari : https://worldwide.promega.com/resources/protocols/product-information-sheets/g/gotaq-green-master-mix-m712-protocol/.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., … Levenez, F. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59–65. doi:10.1038/nature08821 .

Rombout, J. H., Abelli, L., Picchietti, S., Scapigliati, G., & Kiron, V. (2011). Teleost intestinal immunology. *Fish Shellfish Immunol*, *31,* 616–626. doi: 10.1016/j.fsi. 2010.09.001.

Said, N. I. & Marsidi, R. (2001). Mikroorganisme Patogen dan Parasit di dalam Air Limbah Domestik Serta Alternatif Teknologi Pengolahan. *JAI, 1*(1).

Saleem, F., Mustafa, A., Kori, J. A., Hussain, M. S., & Kamran Azim, M. (2018). Metagenomic Characterization of Bacterial Communities in Drinking Water Supply System of a Mega City. *Microbial Ecolog, 76,* 899–910. doi:10.1007/s00248-018-1192-2.

Sambrook, J., Fritsch, E. R., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual (2nd ed.*). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sanders, J. G., Beichman, A. C., Roman, J., Scott, J. J., Emerson, D., McCarthy, J. J., & Girguis, P. R. (2015). Baleen whales host a unique gut microbiome with similarities to both carnivores and herbivores. *Nature Communications*, *6*(1). doi:10.1038/ncomms9285.

Sanger, F., S. Nicklen, dan Coulson, A.R.. (1997). DNA sequencing with chain-terminating inhibitors. *Proc. Nat. Acad. Sci. USA*, *74*(12), 5463–5467. doi:10.1073/pnas.74.12.5463.

Sanschagrin, S., dan Yergeau, E. (2014). Next-generation Sequencing of 16S Ribosomal RNA Gene Amplicons. *Journal of Visualized Experiments,* (90). doi:10.3791/51709.

Sari, I.J., Syamsudin, & Mulis. (2015). Pengaruh Dosis Pakan *Tubifex* Sp Berbedaterhadap Pertumbuhan Benih Ikan Sidatdi Balai BenihIkan Kota Gorontalo. *Nike: Jurnal Ilmiah Perikanan dan Kelautan, 3*(2).

Sarwono, B. (2003). *Budidaya Belut dan Sidat*. Cetakan XXII. (Edisi Revisi). Jakarta: Penebar Swadaya.

Sarwono, B. (2007). *Budidaya Belut dan Sidat*. (Edisi Revisi). Jakarta: Penebar Swadaya.

Sasongko, A., J. Purwanto., S. Mu'minah & U. Arie. (2007). *Sidat, Panduan Penangkapan, Pendederan dan Pembesaran*. Jakarta: Penebar Swadaya.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., … Weber, C. F. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology, 75*(23), 7537–7541. doi:10.1128/aem.01541-09.

Schrezenmeir, J. & de Vrese, M. (2001). Probiotics, prebiotics and symbiotics-approaching a definition. *American Journal of Clinical Nutrition, 73* (suppl): 361S-364S.

Sekirov, I., Russell, S.L, Antunes, L.C., Finlay, B.B. (2010). Gut microbiota in health and disease. *Physiol Rev*., *90*(3), 859-904. doi: 10.1152/physrev.00045.2009.

Sengupta, R., Altermann, E., Anderson, R.C, McNabb, W.C., Moughan, P.J., Roy, N.C. (2013). The role of cell surface architecture of lactobacilli in host- microbe interactions in the gastrointestinal tract. *Mediators Inflamm*, 2013, 1-16.

Setianto, D. (2011). *Cara Mudah dan Cepat Budidaya Sidat*. Jakarta: Pustaka Baru Press.

Shannon, C. E. (1948). A Mathematical Theory of Communication. *Bell System Technical Journal, 27*(3), 379–423. doi:10.1002/j.1538-7305.1948.tb01338.x.

Shendure, J., Mitra, R. D., Varma, C., & Church, G. M. (2004). Advanced sequencing technologies: methods and goals. *Nature Reviews Genetics*, *5*(5), 335–344. doi:10.1038/nrg1325.

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular Ecology, 21*(8), 1794–1805. doi:10.1111/j.1365-294x.2012.05538.x.

Siegerstetter, S.C., Schmitz-Esser, S., Magowan, E., Wetzels, S.U., Zebeli, Q., Lawlor, P.G., O'Connell, N.E., & Metzler-Zebeli, B.U. (2017). Intestinal microbiota profiles associated with low and high residual feed intake in chickens across two geographical locations. *PLoS ONE*, 12(11):e0187766. doi: 10.1371/journal.pone.0187766

Sihombing, D. C., Sasanti, A. D., & Amin, M. (2017). Populasi Bakteri, Efisiensi Pakan, Pertumbuhan Dan Kelangsungan Hidup Ikan Nila (*Oreochromis Niloticus*) yang Diberi Pakan Bersinbiotik. *Jurnal Akuakultur Rawa Indonesia*, *5*(2), 129-139.

Silfani, L. (2018). *Analisis Metagenomik Bakteri pada Usus Ikan sidat (Anguilla sp.) Fase Glass Eel dan Elver*. (Skripsi). Fakultas Pendidikan dan Matematika dan Ilmu Pengetahuan Alam, Universitas Pendidikan Indonesia, Bandung.

Silva, N., Junqueira, V.C.A., & Silveira, N.F.A. (2010). *Manual de métodos de análise microbiológica de alimentos e água. (4. ed.*), São Paulo: Ed. Varela.

Simpson, E.H. (1949). Measurement of diversity. *Nature,* 163: 688.

Soeprijanto, A. Guntur, A. & Fahri, M. (2018). Aplikasi Probiotik dan Pakan Fermentasi pada Pendederan Sidat (*Anguilla bicolor*). *Jurnal Perikanan Universitas Gadjah Mada, 20*(1).

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M. D. M., Breiner, H.-W., & Richards, T. A. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Molecular Ecology*, *19*, 21–31. doi:10.1111/j.1365-294x.2009.04480.x.

Subekti, S., Prawesti, M., & Arief, M. (2011). Pengaruh Kombinasi Pakan Buatan dan Pakan Alami Cacing Sutera (*Tubifex tubifex*) dengan Persentase Yang Berbeda Terhadap Retensi Protein, Lemak Dan Energi Pada Ikan Sidat (*Anguilla bicolor*). *Jurnal KELAUTAN*, *4*(1).

Sugeha, H.Y., S.R. Suharti, S. Wouthuyzen, & K. Sumadhiharga. (2008). Biodiversity, Distribution and Abundance of the Tropical Anguillid Eels in the Indonesian Waters. *Mar. Res. Indonesia*, *33*(2).

Sugiyono. (2016). *Metode Penelitian Kuantitatif, Kualitatif, dan R&D.* Bandung: Alfabeta.

Sulistijowati, S,R., & Mile, L. (2016). Identification of lactic acid bacteria isolates from intestine of milkfish (*chanos-chanos*) potential activity against pathogen bacteria used pcr 18s rrna methode. *International jurnal bio-Science and Bio-Technology*, *8*(3), 127-134.

Suzuki, K., Takemura, M., Miki, T., Nonaka, M., & Harada, N. (2019). Differences in Soil Bacterial Community Compositions in Paddy Fields under Organic and Conventional Farming Conditions. *Microbes and Environments, 34*(1). doi:10.1264/jsme2.me18101.

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., & Nishijima, M. (2014). Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS One*, 9 (8):e105592. https://doi.org/ 10.1371/journal.pone.0007401.

Tasma, I. M. (2015). Pemanfaatan Teknologi Sekuensing Genom Untuk Mempercepat Program Pemuliaan Tanaman. *J. Litbang Pert., 34*(4).

Tavares, A. B., Cereser, N. D., & Timm, C.D. (2015). Occurence of *Aeromonas* spp. in foods of animal origin and its importance in public health. *Arquivos do Instituto Biológico, 82*,1-8.

Thomas, T., Gilbert, J., & Meyer, F. (2012). Metagenomics - a guide from sampling to data. *Microbial Informatics and Experimentation*, 2:3. doi: 10.1186/2042-5783-2-3

Thurkal, A.K. (2017). A review on measurement of Alpha diversity in biology. *Agricultural Research Journal*, *54*(1):1. doi:10.5958/2395-146X.2017.00001.1.

Thursby, E. (2019). Introduction to the human gut microbiota. *Biochemical Journa*, *474*(11), 1823–1836. doi:10.1042/bcj20160510.

Tomás, J. M. (2012). The main *Aeromonas* pathogenic factors. *ISRN Microbiology*, 2012, 256261.

Tsukamoto, K., Nakai, I. & Tesch, W. V. (1998). Do all freshwater eels migrate?. *Nature, 396,* 635–636 .

Tzeng, T.D, Pao, Y.Y., Chen, P.C., Weng, F.C.H, Jean, W.D., Wang, D. (2015) Effects of Host Phylogeny and Habitats on Gut Microbiomes of Oriental River Prawn (*Macrobrachium nipponense*). *PLoS ONE*, 10(7): e0132860. doi:10.1371/journal. pone.0132860.

Valdes, A. M., Walter, J., Segal, E., & Spector, T. D. (2018). Role of the gut microbiota in nutrition and health. *BMJ*, k2179. doi:10.1136/bmj.k2179.

Van Der Meulen, R., Makras, L., Verbrugghe, K., Adriany, T., & Vuyst, L. De. (2006). In Vitro Kinetic Analysis of Oligofructose Consumption by Bacteroides and *Bifidobacterium* spp . *Indicates Different Degradation Mechanisms*. *72*(2), 1006–1012. https://doi.org/10.1128/AEM.72.2.1006

Vasileiadis, S., Puglisi, E., Arena, M., Cappa, F., Cocconcelli, P.S., & Trevisan, M., (2012). Soil bacterial diversity screening using single 16S rRNA gene V regions coupled with multimillion read generating sequencing technologies. *PLoS One*, 7(8):e42671.doi: 10.1371/journal.pone.0042671.

Venter, J.C., S. Levy, T. Stockwell, K. Remington, & A. Halpern. (2003). Massive parallelism, randomness and genomic advances. *Nat. Genet*., *33*, 219–227.

Verma, K., Dalal, J., & Sharma, S. (2014). SCIENTIFIC CONCEPTS OF POLYMERASE CHAIN REACTION (PCR). *International Journal of Pharmaceutical Sciences and Research*, *5*(8), 3086-3095. doi: 10.13040/IJPSR.0975-8232.5(8).3086-95.

Watson, K, A., Kaspar, H., Lategan, M.J., & Gibson, L. (2008). Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture*, *274*(1), 1-14. doi: 10.1016/j.aquaculture.2007.11.019.

Wei, F., Hu, Y., Yan, L., Nie, Y., Wu, Q., & Zhang, Z. (2014). Giant Pandas Are Not an Evolutionary cul-de-sac: Evidence from Multidisciplinary Research. *Molecular Biology and Evolution, 32*(1), 4–12. doi:10.1093/molbev/msu278.

Willemse, J. J. (1979). Guide to the internal morphology of the European eel, *Anguilla anguilla (*L.) (Pisces, Teleostei). *Aquaculture*, *17*(2), 91–103. doi:10.1016/0044-8486(79)90045-0 .

Willis, A. D. (2019). Rarefaction, Alpha Diversity, and Statistics. *Frontiers in Microbiology*, 10. doi:10.3389/fmicb.2019.02407.

Willis, C., Desai, D., & LaRoche, J. (2019). Influence of 16S rRNA variable region on perceived diversity of marine microbial communities of the Northern North Atlantic. *FEMS Microbiology Letters, 366*(13). doi:10.1093/femsle/fnz152.

Wulandari, R,. Rantetondok, A,. Anshary, A,. (2015). "Isolasi bakteri asam laktat dari usus ikan lele untuk pengendalian bakteri *Streptococcu*s pada ikan nila". *Prosiding Simposium Nasional II Kelautan dan Perikanan*. (hlm. 1-10). Makassar: Fakultas Ilmu Kelautan dan Perikanan, UNHAS.

Xia, J. H., Lin, G., Fu, G. H., Wan, Z. Y., Lee, M., Wang, L., … Yue, G. H. (2014). The intestinal microbiome of fish under starvation. *BMC Genomics, 15*(1). https://doi.org/10.1186/1471-2164-15-266.

Xia, L., Xiong, D., Gu, Z., Xu, Z., Chen, C., Xie, J., & Xu, P. (2008). Recovery of Acinetobacter baumannii from diseased channel catfish (Ictalurus punctatus) in China. *Aquaculture, 284*(1-4), 285–288. doi:10.1016/j.aquaculture.2008.07.038.

Xue, Z., Zhang, W., Wang, L., Hou, R., Zhang, M., Fei, L., … Zhang, Z. (2015). The Bamboo-Eating Giant Panda Harbors a Carnivore-Like Gut Microbiota, with Excessive Seasonal Variations. *mBio, 6*(3). doi:10.1128/mbio.00022-15

Yan, Q., van der Gast, C.J., & Yu, Y. (2012) Bacterial Community Assembly and Turnover within the Intestines of Developing Zebrafish. *PLoS ONE*, 7(1): e30603. doi:10.1371/journal.pone.0030603.

Yan, X., Luo, X., & Zhao, M. (2015). Metagenomic analysis of microbial community in uranium-contaminated soil. *Applied Microbiology and Biotechnology, 100*(1), 299–310. doi:10.1007/s00253-015-7003-5.

Yang, W.Y, Lee, Y, Lu, H, Chou, C.H, Wang C. (2019). Analysis of gut microbiota and the effect of lauric acid against necrotic enteritis in *Clostridium perfringens* and *Eimeria* side-by-side challenge model. *PLoS* ONE, 14(5): e0205784. doi: 10.1371/journal.pone.0205784.

Ye, Z., Zhang, N., Wu, C., Zhang, X., Wang, Q., Huang, X., … Yang, P. (2018). *A* metagenomic study of the gut microbiome in Behcet's disease. *Microbiome, 6*(1). doi:10.1186/s40168-018-0520-6.

Yulvizar, C. (2013). Isolasi dan identifikasi bakteri probiotik pada *Rastrelliger* sp. *Jurnal biologi, 6*(2), 1-7.

Zhou, X., Wang, Y., Yao, J., & Li, W. (2010) Inhibition alibilty of probiotic, *Lactotococcus lactis*, against A. Hydrophila and study of its imunostimulatory effect in tilapia (*Oreocromis niloticus*). *International Journal of Engineering Science and Technology, 2*(7). doi: 10.4314/ijest.v2i7.6374.

Zhou, M., Liang, R., Mo, J., Yang, S., Gu, N., Wu, Z., … Lin, L. (2018). Effects of brewer’s yeast hydrolysate on the growth performance and the intestinal bacterial diversity of largemouth bass (*Micropterus salmoides*