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DNA Barcoding and Water Quality Analysis of Nitrifying Bacteria in Lebak Lebung Swamp, South Sumatera

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ABSTRACT

Aquaculture development activities in swamp water has the problem of contamination from organic matter, and this waste has the potential for environmental challange. Nitrifying bacteria are a natural instrument that can play a role in maintaining the stability of the quality of swamp waters through their role as bioremediator. Therefore, its presence is important to identify in waters. The aim of the research is to determine the types and characteristics of bioremediation bacteria, construct a phylogenetic tree and the relationship between water quality and the bioremediation process by bacteria so that in the future it can be applied to waters that have the same problems or become a bioindicator for certain pollutants, especially in the area of Lebak Lebung Swamp, Ogan. Ilir, South Sumatra. The method used is taking bacterial samples, isolating bacteria using Nutrient Agar (NA) media, observing bacterial morphology, DNA sequencing, amplifying DNA mitochondria COI using PCR (Polymerase Chain Reaction). The results of BLASTn (Basic Local Alignment Search Tool-nucleotide) analysis showed the highest percentage of identity, namely 93%, with the Burkholderia cepacia strain NBRAJG97 from India and Burkholderia sp. strain 172 1492R comes from Estonia which indicates that the bacteria found belong to the Burkholderia bacteria group. Water quality measurement was temperature 34.7-35.4°C, dissolved oxygen 6.0-6.2 mgL^{-1}, pH 6.2, ammonia 0.05 mgL^{-1}. Based on air quality indications such as low ammonia content, this could indicate that the Burkholderia bacteria found in Lebak Lebung Swamp play a role as bioremediation.

1. INTRODUCTION

Nitrification will follow the ammonification process, which is a process of reforming organic nitrogen from land waste into ammonium [1, 2]. The nitrification process will be followed by an increase in organic nitrogen due to the abundance of landfill being a concern of many scientists because the large amount of organic nitrogen will have negative implications on water quality, including eutrophication or increased organic biomass [3-5]. The nitrification process is played by the bacteria Nitrosomonas sp. which converts ammonia into nitrites [6] and the bacteria Nitrobacter sp. which will oxidize nitrites to nitrates [7, 8]. This nitrate is harmless to fish life and tends to be used by aquatic plants to grow and develop [9]. The influence of Nitrosomonas and Nitrobacter bacteria is very important in reducing nitrite and increasing nitrate in fish farming [10-12]. The problems that occur in the field are that the nitrification process does not work properly, causing the buildup of ammonia which will result in the death of the fish. The main cause is the lack of nitrifying bacteria in helping to decompose organic matter found in aquaculture ponds.

Nitrite is found in the waters in very small amounts compared to nitrates, because it is not stable in the presence of oxygen [13]. Nitrate is a source of nitrogen for plants which is then converted to protein. Nitrates are very easy to dissolve in water and are stable [14-16]. Organic nitrogen in waters is sourced from waste disposal from the land including household activities, industry, fertilization and cultivation which triggers an increase in the amount of organic material entering the waters [17-19]. The nitrification process in aquaculture ponds is very important with the help of Nitrosomonas and Nitrobacter bacteria, the help of these two genera of bacteria can reduce the ammonia content contained in aquaculture ponds and can increase the fish production process [20-22]. This prompted the need for studies related to the isolation of nitrifying bacteria as probiotic agents to overcome various problems of fish mortality [22]. One of the first steps that can be taken is to screen and isolate nitrifying

bacteria.

Previous studies have succeeded in isolating nitrifying bacteria from various cultivation media, such as research conducted by Aswiyanti et al. [23] who isolated nitrifying bacteria from tilapia cultivation media and succeeded in obtaining bacterial isolates of the type *Klebsiella* spp. This was further explained by Hastuti et al. [24] who isolated nitrifying bacteria from mud crab cultivation media using a recirculation system or water flowing continuously and succeeded in obtaining isolates of *Pseudomonas stutzerii* and *Halomonas* sp. Currently there is no research that has isolated nitrifying bacteria in swamp waters so it is possible that the bacteria obtained are local bacteria which can later be used as probiotics for fish farming in swamp waters. This is of course very important for the sustainability of cultivation in peat swamp waters.

Isolation, selection, and characterization are important steps to get the desired bacteria. The stages of the process are needed for the type of bacteria obtained has the ability according to the target. The characterization stage was carried out to determine the type of bacteria and its kinship relationship using 16S rRNA sequencing. Taxonomic analysis using 16S and 18S rRNA genes for sequencing data has shown a valid approach in characterizing bacterial communities [25, 26]. Aquaculture development activities in swamp water or wetlands that are inundated with water at certain periods of time has the problem of contamination from organic matter, and this waste has the potential as a nitrogen source in swamp water. Therefore, research on the isolation and characterization of nitrifying bacteria in swamp water to be identified. By identifying bacteria that have a role as bioremediators, this can become a tool that can be used to determine the condition of a particular body of water. This is because the presence of these bacteria indicates a process of change that occurs naturally in the waters in order to maintain the stability of environmental conditions and is an illustration that there is pollution/waste contained in these waters.

2. MATERIALS AND METHODS

2.1 Research place

This research was conducted at the Laboratory of Microbiology at Department of Mathematic and Natural Science, and Laboratory of Aquaculture and Plant Physiology at Department of Agriculture Cultivation Faculty of Agriculture Sriwijaya University.

2.2 Sample description

Samples are stored in a watertight and clean container. All tools and materials as well as the laboratory environment have been ensured to be sterile to avoid external contamination. Soil and air samples from the Lebak Lebung Ogan Ilir swamp waters were taken at the same point, namely the edge. Taking soil samples at the edge makes it easier to take the amount of soil compared to taking the middle part of the water. According to Kusmawati [27], soil is one of the growing media of various kinds of animals, microbes, because it has a complex source of nutrients for bacterial growth. Water quality measurements include temperature using thermometer, pH using pH meter, dissolved oxygen using DO meter and ammonia using a spectrophotometer.

2.3 Isolation of bacteria candidates

Isolation of nitrifying bacteria using the enrichment culture method was carried out by inoculating 1 gram of the soil sample obtained into each NA (Nutrient Agar) media. The liquid culture containing the isolate was homogenized with a shaker for 7 days or until a color change occurred. The presence of ammonium-oxidizing bacteria is indicated by a change in the color of the media from red to yellow due to changes in the pH of the media due to the oxidation of ammonium to nitrite, while the presence of nitrate-producing bacteria is indicated by changes in the media. color from clear to cloudy [28, 29]. Next, the bacterial colonies that grow on certain media in petri dishes are taken one by one and planted back in the dish to be purified by incubating at room temperature (27-28)℃ in anaerobes conditions.

2.4 Observation of colony morphology

The pure bacterial isolate was then identified through two observations, namely: a) observing the morphology of the bacterial isolate which was observed including color, cell shape, edges and surface, b) microscopic observation by testing the gram characteristics of the isolate using differential staining and observing through a microscope with 100× magnification to know the gram test reaction [30, 31].

2.5 DNA extraction

The bacterial DNA extraction process was carried out using PrestoTM Mini gDNA Bacteria Kit (Geneaid Biotech Ltd., City, Country). The sample required 1 × 106 bacterial cell for one extraction. The DNA extraction procedure was carried out in accordance with the manual PrestoTM Mini gDNA Bacteria Kit.

2.6 Amplification and electrophoresis

The PCR material was used 25 µl of DNA extraction. Each reaction contains: go taq green 12.5 µl, there are two pairs of 16S rRNA primers namely 63f primer 1 µl, primer 1387r 1 µl, NFW (Nuclease Free Water) 6.5 µl, DNA template 4 µl. DNA amplification is carried out by stages: initiation cycle at 95℃ for 5 minutes, denaturation at 94℃ for 30 seconds, annealing or primary attachment at 50℃ for 1 minute, extension or elongation at 72℃ for 2 minutes in 30 cycles and the final extension at 72℃ for 5 minutes [32]. PCR products are electrophoresed through 1% agarose electrophoresis gel. 1 µL of dye loading was mixed with 6 µL of DNA inserted in each well electrophoresis. Electrophoresis was carried out with 75V power for 50 minutes and the results were immersed into TAE 1X which had been added with diamond nucleic acid dye for 30 minutes, then visualized with Gel Doc. The DNA size of the Gel Doc results using a 1 kb marker. The results of the amplification of known DNA sizes using electrophoresis were then sequenced.

2.7 Data analysis

Sequences that have been obtained in the form of fasta format are then aligned using MEGA 6.0 software, then sequenced sequences are taken for later BLAST (Basic Local Alignment Search Tool) which is used to determine the homology of a DNA or amino acid sequence with the data

contained in Genbank NCBI (National Center for Biotechnology Information) and Barcode of Life. Furthermore, all sequences are aligned for genetic distance and phylogenetic trees. Phylogenetic trees between bacterial species were constructed using the Maximum Likelihood method.

3. RESULTS AND DISCUSSION

3.1 Isolation and characteristics morphology of bacteria candidates

The results of bacterial isolation obtained in the swampy waters of the swamp obtained a total of 10 isolates, but based on the characteristic equation of each isolate only one isolate was selected for further testing because it has similarities in morphological characteristics. The isolate with the following morphological characteristics (Table 1):

Table 1. Morphological characteristics

Morphology of Selected Isolates Bacteria	
Colour	Brownish
Shape of cell	Basil
Edge	Flat
Surface	Convex
Gram reaction	Negative (-)

The tested bacteria were pure isolates of nitrifying bacteria that had the N1 code. The location of sampling of land and water is carried out in 2 places, namely on the right side and the left side of the edge. According to Kusmawati [27], soil is one of the growing media of various types of organisms, one of them, microbes, because it has a complex source of nutrients for bacterial growth. The results of the morphological characteristics have not been able to provide information about the genus from the isolates that have been obtained. Therefore, further testing of these isolates was carried out.

3.2 Molecular characteristics of bacterial isolates

The amplification or multiplication of target DNA is intended to increase the amount of target DNA using the PCR method with universal bacterial primers namely 16S rRNA 63F (Forward) and 1387R (Reverse) and the template of DNA extraction of 4 µL nitrifying bacteria can be detected by DNA visualization. DNA visualization is presented in Figure 1.

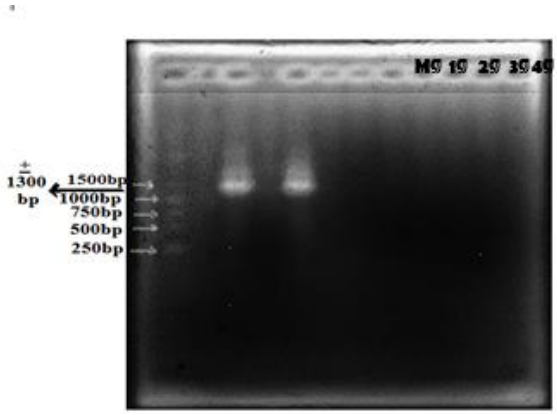

Figure 1. Visualization of 16S rRNA gene isolation results from bacterial isolates
M= Marker

Based on Figure 1 visualization of the results of amplification of 16S rRNA in ± 1300 bp bacterial isolates. According to Marchesi et al. [33] in practice 63f and 1387r primers were more successful than amplimer 27f-1392r pairs and reinforced 16S rRNA genes from a variety of bacteria that were better than others which are usually used for analysis of bacterial communities. Primary 63f and 1387r have been evaluated to amplify the 16S rRNA gene from Domain bacteria. This primary pair is able to amplify the gene with a size of about 1300 base pairs. The visualization process is one way to determine the quality of DNA isolates and ensure the suitability of primers used by looking at the length of base pair (bp) [34].

The sequenced results are then aligned using Mega 6.0 software. After aligning produce a nucleotide base sequence with a length of 1380 bp. Furthermore, isolates were analyzed by BLAST (Basic Local Alignment Search Tool-nucleotide) at the NCBI website (National Center for Biotechnology Information) showed the results of the percentage similarity of samples of nitrifying bacteria with data found in Genbank. BLAST analysis of nucleotide sequences of 16S rRNA gene samples of nitrifying bacteria isolates N1 had the highest percentage of identity, 93% with Indian species Burkholderia cepacia strain NBRAJG97 and Burkholderia sp. strain 172 1492R originating from Estonia. According to Syakti et al. [35] if homology is more than 97% of the same species, between 93% and 97% the genus is the same but different species, and if it is smaller than 93% it is likely a new species. Usually, a method based on this philosophy is known as the distance method [36]. Genetic distance was analyzed using Pairwise Distances with the Maximum Composite Likelihood and Substitutions models to included: Transitions + Transversions in MEGA 6.0 and the results of genetic distance analysis of nitrifying bacteria showed that isolates observed in the study were N1 isolates having the lowest genetic distance of 0.004 (0, 4%) with the *Burkholderia cepacia* strain of NBRAJG97 (EU734821.1) from India.

The level of genetic similarity of a population can be described by genetic distance from individual members of the population. The smaller the genetic distance between individuals in a population, the more uniform the population will be. Conversely the greater the genetic distance of individuals within a population, then the population has increasingly diverse members [37].

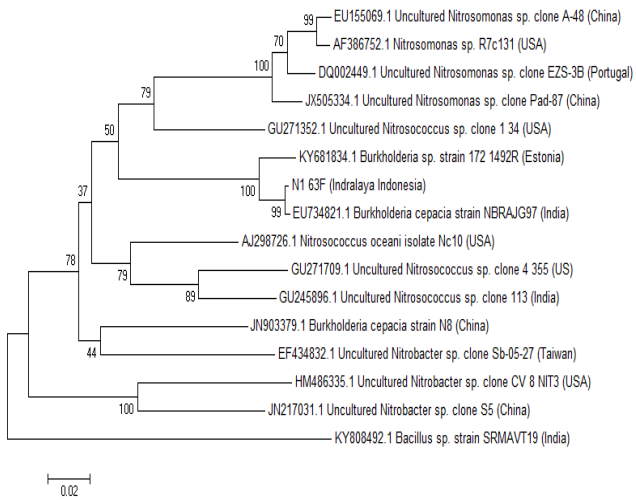

Figure 2. Phylogenetic tree of nitrifying bacteria

The kinship of an organism can be described through phylogenetic trees which are constructed based on genetic distance analysis. The phylogenetic tree is a two-dimensional graph that shows the relationship between organisms or population classifications based on the history of their evolution. Phylogenetic trees are constructed through the Mega 6.0 software application using the Neighbor-Joining (NJ) method [38] of the Maximum Composite Likelihood and Substitutions models to included: Transitions + Transversions with 1000× boostrap [39, 40]. The eubacteria phylogenetic tree is presented in Figure 2.

The results of phylogenetic tree construction showed that nitrifying bacteria formed branches with a scale of 0.02. The phylogenetic tree of nitrifying bacteria showed that the sample of N1 isolate had a 99% bootstrap value with the species Burkholderia cepacia strain NBRAJG97 from India, this was seen in the species forming its own subcluster. Member of genus Burkholderia are common soil inhabitants and that their biogeographic distribution is strongly affected by soil Ph. Burkholderia strains have a competitive advantage in acidic soils but are outcompeted in alkaline soils [41]. Burkholderia cepacia can be classified as follows: Divisio Proteobacteria; Betaproteo bacteria class; Order of Burkholderiales; Burkholderiaceae family; Burkholderia genus [42]. B. cepacia is still genomovar (one genetic species that is closely related) with Pseudomonas, namely P. kingii, P cepacia, P. multivorans. Burkohlderia cepacia bacteria are heterotrophic nitrifying bacteria that are able to convert nitrite (NO_2-) into nitrate (NO3-) which then converts nitrite (NO_2-) into NO with the help of the nitrite reductase enzyme which plays a role in reducing nitrite concentrations. Next, the nitrogen that has been formed is further converted into nitrate (NO_3-) by the dioxygenase enzyme under aerobic conditions [43-45].

Research conducted by Gohar et al. [46] stated that the Burkholderia cepacian type of bacteria showed strong activity against Aeromonas hydrophila, Edwardsiella tarda, and Vibrio ordalli. The results of the rough extraction of these bacteria produce antimicrobial fractions. They are (1) Phenol, (2) Phenol-4-methyl, (3) 3-benzyl-1,4-diaza-2,5-dioxobicyclo (4) hexadecanoic acid ethyl ester, and (5) 1,2 Benzene acid decarboxylated. Further explained by Young et al. [47]. which stated that Burkholderia sp. CC-Al74 can increase the level of P utilization and the total P and N content after endophytic colonization. The Burkholderia genus is rich in nitrogen-fixing and phosphate-solubilizing strains [48]. Burkholderia produces a structural array of specific metabolites with antifungal and antibacterial properties. In addition, Burkholderia is a relatively untapped resource for the discovery of natural antimicrobial products with diverse cellular targets and has promising potential for clinical, agricultural and fisheries uses [49]. Burkholderia β-Proteobacteria is a promising source of NP, development of Burkholderia bacteria as heterologous hosts and application of Burkholderia in industrial-scale NP production [50-55].

3.3 Water quality

The results of measurement of water quality at the location where sampling is shown in Table 2.

Measurements of water quality observed at the location of sampling included parameters of temperature, pH, DO and ammonia. The measured temperature has a fairly high value, this is because temperature measurements are carried out during the day, which according to Nelson et al. [51] the amount of heat from sunlight entering the waters or its spread is greater so it can affect the media. In general, the swamp waters are somewhat acidic to neutral (pH 4 to neutral) with more acidic tendencies during the dry season [41].

Table 2. Water quality at the location of sampling

No.	Water Quality Parameters	Station
1.	Suhu (°C)	34.7 – 35.4
2.	pH	6.21
3.	DO ($mg.L^{-1}$)	6.0 – 6.2
4.	Amonia ($mg.L^{-1}$)	0.05

In general, DO has a tendency that is directly proportional to depth [42]. This is caused increasingly towards the base of air diffusion and photosynthetic activity decreases, and oxygen reduction due to decomposition of organic matter in the water base is getting bigger [43]. The ammonia content in swamp water is 0.05 mgL^{-1}. Based on Marbun et al. [55], un-ionized free ammonia levels should be no more than 0.2 mgL^{-1}. Based on the water quality conditions obtained, the conditions obtained are still considered optimal for the growth of fish and bacteria. There are water quality exceptions to bacterial populations because they have a good ability to withstand even extreme environmental conditions.

4. CONCLUSION

Burkohlderia bacterium identified in the waters of Lebak Lebung Swamp, Ogan Ilir, South Sumatra are known to have a role as nitrifying bacteria. In the future, they can be applied as bioremediators in mass cultivation activities and in their natural conditions can also be bioindicators of the condition of balance in the aquatic environment.

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REFERENCES

[1] Samarasinghe, S.A.P.L., Shao, Y., Huang, P.J., Pishko, M., Chu, K.H., Kameoka, J. (2016). Fabrication of bacteria environment cubes with dry lift-off fabrication process for enhanced nitrification. PLoS ONE, 11(11): e0165839. https://doi.org/10.1371/journal.pone.0165839

[2] Van Kessel, M.A., Speth, D.R., Albertsen, M., et al. (2015). Complete nitrification by a single microorganism. Nature, 528(7583): 555-559. https://doi.org/10.1038/nature16459

[3] Ayiti, O.E., Babalola, O.O. (2022). Factors influencing soil nitrification process and the effect on environment and health. Frontiers in Sustainable Food Systems, 6(2): 821994. https://doi.org/10.3389/fsufs.2022.821994

[4] Yan, Y., Lu, H., Zhang, J., Zhu, S., Wang, Y., Lei, Y., Zhang R., Song, L. (2022). Simultaneous heterotrophic nitrification and aerobic denitrification (SND) for

nitrogen removal: A review and future perspectives. Environmental Advances, 9(11): 100254. https://doi.org/10.1016/j.envadv.2022.100254

[5] Amiin, M.K., Lahay, A.F., Putriani, R.B., Reza, M., Putri, S.M.E., Sumon, M.A.A., Jamal, M.T Santanumurti, M.B. (2023). The role of probiotics in vannamei shrimp aquaculture performance–A review. Veterinary World, 16(3): 638. https://doi.org/10.14202/vetworld.2023.638-649

[6] Rodríguez, A.R., Inchaustegui, S.M., Castro, L. P., Montealegre, R.J., Vargas, J.P.H. (2017). Isolation of ammonium- and nitrite-oxidizing bacterial strains from soil, and their potential use in the reduction of nitrogen in household waste water. Revista de Biología Tropical, 65(4): 1527. https://doi.org/10.15517/rbt.v65i4.26509

[7] Cai, M., Ng, S.K., Lim, C.K., Lu, H., Jia, Y., Lee, P.K. (2018). Physiological and metagenomic characterizations of the synergistic relationships between ammonia- and nitrite-oxidizing bacteria in freshwater nitrification. Frontiers in Microbiology, 9(2): 280. https://doi.org/10.3389/fmicb.2018.00280

[8] Taragusti, A.S., Santanumurti, M.B., Rahardja, B.S. Prayogo. (2019). Effectiveness of *Nitrobacter* on the specific growth rate, survival rate and feed conversion ratio of dumbo catfish *Clarias* sp. with density differences in the aquaponic system. IOP Conference Series: Earth and Environmental Science, 236(1): 012088. https://doi.org/10.1088/1755-1315/236/1/012088

[9] Atique, F., Lindholm-Lehto, P., Pirhonen, J. (2022). Is aquaponics beneficial in terms of fish and plant growth and water quality in comparison to separate recirculating aquaculture and hydroponic systems? Water, 14(9): 1447. https://doi.org/10.3390/w14091447

[10] Ramdhani, N., Kumari, S., Bux, F. (2013). Distribution of nitrosomonas related ammonia-oxidizing bacteria and nitrobacter related nitrite-oxidizing bacteria in two full-scale biological nutrient removal plants. Water Environment Research, 85(4): 374-381. https://doi.org/10.2175/106143013x13596524516022

[11] Setiawan, D., Prayogo., Rahardja, B.S. (2021). Utilization of *Nitrosomonas* sp. and *Nitrobacter* sp. probiotic towards nitrite and nitrate level in nile tilapia (*Oreochromis niloticus*) using aquaponic system. IOP Conference Series: Earth and Environmental Science, 718(1): 012098. https://doi.org/10.1088/1755-1315/718/1/012098

[12] Nurhasanah, A., Rahardja, B.S. Prayogo. (2023). Effect of probiotics in recirculating aquaculture system (RAS) on the concentration of ammonia, nitrite, and nitrate in the aquaculture of catfish (*Clarias* sp.). IOP Conference Series: Earth and Environmental Science, 1273(1): 012018. https://doi.org/10.1088/1755-1315/1273/1/012018

[13] Putri, A.O., Pamula, O.Y.T., Fakhriah, Y., Prayogo., Sudarno., Manan, A., Sari, L.A., Dewi, N.N. (2019). The comparison of water spinach (*Ipomoea aquatica*) density using aquaponic system to decrease the concentration of ammonia (NH3), nitrite (NO2), nitrate (NO3) and its effect on feed conversion ratio and feed efficiency to increase the survival rate and specific growth rate of African catfish (*Clarias* sp.) in intensive aquaculture. Journal of Aquaculture and Fish Health, 8(2): 113-122. https://doi.org/10.20473/jafh.v8i2.13626

[14] Lubis, L.H., Husin, A., Masyitah, Z. (2022). Nitrate (NO_3^-) removal from wastewater by adsorption using modified kaolin. IOP Conference Series: Earth and Environmental Science, 963(1): 012038. https://doi.org/10.1088/1755-1315/963/1/012038

[15] Pottinger, T.G. (2017). Modulation of the stress response in wild fish is associated with variation in dissolved nitrate and nitrite. Environmental Pollution, 225: 550-558. https://doi.org/10.1016/j.envpol.2017.03.021

[16] Nkuba, A.C., Mahasri, G., Lastuti, N.D.R., Mwendolwa, A.A. (2021). Correlation of nitrite and ammonia concentration with prevalence of Enterocytozoon hepatopenaei (EHP) in shrimp (*Litopenaeus vannamei*) on several super-intensive ponds in East Java, Indonesia. Jurnal Ilmiah Perikanan dan Kelautan, 13(1): 58-67. https://doi.org/10.20473/jipk.v13i1.24430

[17] Golterman, H.L. (2004). The Chemistry of Phospate and Nitrogen Compounds in Sediments. Kluwer Academic Publishers.

[18] Moloantoa, K.M., Khetsha, Z.P., Van Heerden, E., Castillo, J.C., Cason, E.D. (2022). Nitrate water contamination from industrial activities and complete denitrification as a remediation option. Water (Switzerland), 14(5): 799. https://doi.org/10.3390/w14050799

[19] Wijaya, I., Soedjono, E.S., Fitriani, N. (2017). Development of anaerobic ammonium oxidation (anammox) for biological nitrogen removal in domestic wastewater treatment (Case study: Surabaya City, Indonesia). AIP Conference Proceedings, 1903(1). https://doi.org/10.1063/1.5011532

[20] Widigdo, B., Yuhana, M., Iswantari, A., Madonsa, C., Sapitri, I.D., Wardiatno, Y., Hakim, A.A., Nazar, F. (2021). The impact of nitrifying probiotic to population growth of pathogenic bacteria, *Vibrio* sp., and toxic nitrogen gasses in marine shrimp culture media under laboratory condition. Journal of Natural Resources and Environmental Management, 11(1): 130-140. https://doi.org/10.29244/jpsl.11.1.130-140

[21] Lukmantoro, T.A., Prayogo., Rahardja, B.S. (2020). Effect of different filter media use on aquaponics system on ammonium (NH4+), nitrite (NO2) and nitrate (NO3) concentrations of catfish (Clarias sp.) aquaculture. IOP Conference Series: Earth and Environmental Science, 441: 012121. https://doi.org/10.1088/1755-1315/441/1/012121

[22] Dwiardani, K.H., Prayogo., Rahardja, B.S. (2021). Utilization of *Nitrosomonas* sp and *Nitrobacter* sp probiotic towards total suspended solid and ammonia level in nile tilapia culturing using aquaponic system. IOP Conference Series: Earth and Environmental Science, 679(1): 012067. https://doi.org/10.1088/1755-1315/679/1/012067

[23] Aswiyanti, I., Istiqomah, I., Isnansetyo, A. (2021). Isolation and identification of nitrifying bacteria from tilapia (*Oreochromis* sp.) pond in Sleman Yogyakarta Indonesia. IOP Conference Series: Earth and Environmental Science, 919(1): 012054. https://doi.org/10.1088/1755-1315/919/1/012054

[24] Hastuti, Y.P., Rusmana, I., Nirmala, K., Affandi, R., Tridesianti, S. (2019). Short Communication: Identification and characterization of nitrifying bacteria in mud crab (*Scylla serrata*) recirculation aquaculture system by 16S rRNA sequencing. Biodiversitas, 20(5):

1339-1343. https://doi.org/10.13057/biodiv/d200524
[25] Ciuffreda, L., Rodríguez-Pérez, H., Flores, C. (2021). Nanopore sequencing and its application to the study of microbial communities. Computational and Structural Biotechnology Journal, 19: 1497-1511. https://doi.org/10.1016/j.csbj.2021.02.020
[26] Zheng, J.J., Wang, P.W., Huang, T.W., Yang, Y.J., Chiu, H.S., Sumazin, P., Chen, T.W. (2022). MOCHI: a comprehensive cross-platform tool for amplicon-based microbiota analysis. Bioinformatics, 38(18): 4286-4292. https://doi.org/10.1093/bioinformatics/btac494
[27] Kusmawati I. (2013). Isolation of nitrifying bacteria in the rhizospheric area of mandoti pulu local rice (*Oryza Sativa* L.) in Salukanan Village, Enrekang Regency, South Sulawesi. Hasanuddin University Makassar.
[28] Bhusal, A., Muriana, P.M. (2021). Isolation and characterization of nitrate reducing bacteria for conversion of vegetable-derived nitrate to 'natural nitrite.' Applied Microbiology, 1(1): 11-23. https://doi.org/10.3390/applmicrobiol1010002
[29] Rosier, B.T., Moya-Gonzalvez, E.M., Corell-Escuin, P., Mira, A. (2020). Isolation and characterization of nitrate-reducing bacteria as potential probiotics for oral and systemic health. Frontiers in Microbiology, 11(11): 555465. https://doi.org/10.3389/fmicb.2020.555465
[30] Ayal, E.L.B., Kasprijo, K., Fitriadi, R., Ryandini, D., Nurhafid, M., Riady, R.M., Anandasari, M.A. (2024). Screening of amylolytic bacteria from mina Padi aquaculture in Panembangan Village, Cilongok District, Banyumas, Central Java: Screening of Amylolytic Bacteria from Mina Padi Aquaculture. Journal of Aquaculture and Fish Health, 13(1): 90-101. https://doi.org/10.20473/jafh.v13i1.39210
[31] Becerra, S.C., Roy, D.C., Sanchez, C.J., Christy, R.J., Burmeister, D.M. (2016). An optimized staining technique for the detection of Gram positive and Gram negative bacteria within tissue. BMC Research Notes, 9(1): 216. https://doi.org/ 10.1186/s13104-016-1902-0
[32] Iweriebor, B.C., Obi, L.C., Okoh, A.I. (2015). Virulence and antimicrobial resistance factors of Enterococcus spp. isolated from fecal samples from piggery farms in Eastern Cape, South Africa Ecological and evolutionary microbiology. BMC Microbiology, 15(1): 136. https://doi.org/10.1186/s12866-015-0468-7
[33] Marchesi, J.R., Sato, T., Weightman, A.J., Martin, T.A., Fry, J.C., Hiom, S.J., Wade, W.G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Applied and Environmental Microbiology, 64(2): 795-799. https://doi.org/10.1128/AEM.64.2.795-799.1998
[34] Riyantini, I., Mulyani, Y., Agung, M.U.A. (2014). Molecular phylogenetics relationship among several mangroves in Penjarangan island, Ujung Kulon, Banten province. Jurnal Akuatika Indonesia., 5(1): 245336.
[35] Syakti, A.D., Lestari, P., Simanora, S., Sari, L.K., Lestari, F., Idris, F., Agustiadi, T., Akhlus, S., Hidayati, N.V., Riyanti. (2019). Culturable hydrocarbonoclastic marine bacterial isolates from Indonesian seawater in the Lombok Strait and Indian Ocean. Heliyon, 5(5): e01594. https://doi.org/10.1016/j.heliyon.2019.e01594
[36] Zein, M.S.A., Prawiradilaga, D.M. (2013). DNA Barcoding Fauna Indonesia. Kencana Press.
[37] Trisyani, N., Rahayu, D.A. (2020). DNA barcoding of razor clam solen spp. (solinidae, bivalva) in Indonesian beaches. Biodiversitas Journal of Biological Diversity, 21(2): 478-484. https://doi.org/10.13057/biodiv/d210207
[38] Xia, K., Li, Y., Sun, J., Liang, X. (2016). Comparative genomics of Acetobacterpasteurianus Ab3, an acetic acid producing strain isolated from Chinese traditional rice vinegar Meiguichu. PLoS ONE, 11(9): e0162172. https://doi.org/10.1371/journal.pone.0162172
[39] Wijayanti, M., Jubaedah, D., Suhada, J.A., Yuliani, S., Saraswati, N., Syaifudin, M., Widjajanti, H. (2018). DNA barcoding of swamp sediment bacterial isolates for swamp aquaculture probiotic. E3S Web of Conferences, 68: 01023. https://doi.org/10.1051/e3sconf/20186801023
[40] Wijayanti, M., Syaifudin, M., Yulisman, Y., Nurianti, Y., Hidayani, A., Gofar, N. (2020). Characterization of arthrospira platensis cultured in wastewater of clarias catfish farming media: Dna barcode, helical form, growth, and phycocyanin. Biodiversitas, 21(12): 5872-5883. https://doi.org/10.13057/biodiv/d211252
[41] Stopnisek, N., Bodenhausen, N., Frey, B., Fierer, N., Eberl, L., Weisskopf, L. (2014). Genus-wide acid tolerance accounts for the biogeographical distribution of soil Burkholderia populations. Environmental Microbiology, 16(6): 1503-1512. https://doi.org/10.1111/1462-2920.12211
[42] Nursyirwani., Amolle, K.C. (2007). Isolation and characterization of hydrocarbonoclastic bacteria from dumai waters with 16S rDNA Sequences. Journal of Marine Sciences, 12(1): 12-17.
[43] He, T., Xie, D., Ni, J., Li, Z., Li, Z. (2020). Nitrous oxide produced directly from ammonium, nitrate and nitrite during nitrification and denitrification. Journal of Hazardous Materials, 388(12): 122114. https://doi.org/10.1016/j.jhazmat.2020.122114
[44] Matsuzaka, E., Nomura, N., Maseda, H., Otagaki, H., Nakajima-Kambe, T., Nakahara, T., Uchiyama, H. (2003). Participation of nitrite reductase in conversion of NO2- to NO3- in a heterotrophic nitrifier, burkholderia cepacia NH-17, with denitrification activity. Microbes and Environments, 18(4): 203-209. https://doi.org/10.1264/jsme2.18.203
[45] Rosenberg, E. (2014). The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Springer Reference. https://doi.org/10.1007/978-3-642-30197-1
[46] Gohar, Y.M., El-Naggar, M.M., Soliman, M.K., Barakat, K.M. (2010). Characterization of marine Burkholderia cepacia antibacterial agents. Journal of Natural Products, 74: 86-94.
[47] Young, L.S., Hameed, A., Peng, S.Y., Shan, Y.H., Wu, S.P. (2013). Endophytic establishment of the soil isolate Burkholderia sp. CC-Al74 enhances growth and P-utilization rate in maize (Zea mays L.). Applied Soil Ecology, 66: 40-47. https://doi.org/10.1016/j.apsoil.2013.02.001
[48] Muthukumarasamy, R., Revathi, G., Vadivelu, M., Arun, K. (2017). Isolation of bacterial strains possessing nitrogen-fixation, phosphate and potassium-solubilization and their inoculation effects on sugarcane. Indian Journal of Experimental Biology, 55(3): 161-170. https://doi.org/10.14710/ik.ijms.12.1.12-17
[49] Kunakom, S., Eustáquio, A.S. (2020). Burkholderia as a source of natural products sylvia. Encyclopedia of Marine Biotechnology. 82(7): 2147-2160.

https://doi.org/10.1002/9781119143802.ch96
[50] Adaikpoh, B.I., Fernandez, H.N., Eustáquio, A.S. (2022). Biotechnology approaches for natural product discovery, engineering, and production based on Burkholderia bacteria. Current Opinion in Biotechnology, 77: 102782. https://doi.org/10.1016/j.copbio.2022.102782
[51] Nelson, K.L., Boehm, A.B., Davies-Colley, R.J., et al. (2018). Sunlight-mediated inactivation of health-relevant microorganisms in water: A review of mechanisms and modeling approaches. Environmental Science: Processes & Impacts, 20(8): 1089-1122. https://doi.org/10.1039/C8EM00047F
[52] Welcomme, R.L. (1979). Fisheries Ecology of Floodplain Rivers. Longman Inc.
[53] Aida, S.N., Utomo, A.D. (2017). Kajian kualitas perairan untuk perikanan di rawa pening Jawa Tengah. BAWAL Widya Riset Perikanan Tangkap, 8(3): 173-182. https://doi.org/10.15578/bawal.8.3.2016.173-182
[54] Leidonald, R., Muhtadi, A., Lesmana, I., Harahap, Z.A., Rahmadya, A. (2019). Profiles of temperature, salinity, dissolved oxygen, and pH in Tidal Lakes. IOP Conference Series: Earth and Environmental Science, 260(1): 012075. https://doi.org/10.1088/1755-1315/260/1/012075
[55] Marbun, R., Gulton, J. (2022). Study on utilization of active natural zeolite as ammonia absorbent in aquarium as a medium fresh fish cultivation. Journal of Chemical Natural Resources, 4(1): 44-48. https://doi.org/10.32734/jcnar.v4i1.9358