Journal of Advanced Zoology

ISSN: 0253-7214

Volume ***44*** *Issue* ***02 Year 2023*** *Page* ***191:199***

Isolation and Characteristics of Bioflocculant Producing Bacteria from Vannamei Shrimp Ponds

Petrus Hary Tjahja Soedibya[1], Agung Cahyo Setyawan[1], Mustika Palupi[1], Ren Fitriadi[1*], Mohammad Nurhafid[1], Reza Muhammad Riady[1]

[1]Aquaculture Study Program, Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University St. Dr. Soeparno, Karangwangkal, Purwokerto 53122, Central Java, Indonesia.

**Corresponding author's E-mail: renfitriadi@unsoed.ac.id*

Article History

Received: 06 June 2023
Revised: 25 Sept 2023
Accepted:01Oct 2023

Abstract

Developing fish farming technology is an urgent research topic to increase fish farming productivity. Global aquaculture production generated about 50 million tons of fish, in 2020, fishery production reached 214 million tons or IDR 424 billion. Aquatic biota production in 2020, 60% higher than the 1990 average, far exceeded world population growth, largely due to increased aquaculture production. One of the technologies used to increase shrimp aquaculture production is biofloc technology. The main aim of this study was to isolate and briefly characterize bioflocculant bacteria from the Vannamei shrimp pond habitat. Research methods included collecting bacteria, isolating bioflocculant-producing bacteria, purifying bacteria, testing bacterial flocculant activity, and identifying bioflocculant bacteria using 16s rDNA. The results showed that 5 isolates showed the highest bioflocculant activity, namely 20.9-44.40%. The five isolates were identified as Vibrio Navarensis, Staphylococcus gallinarum, Pseudoalteromonas ganghwensis, and Cytobacillus kochii. The highest flocculant activity value was obtained by the bacteria Pseudoalteromonas ganghwensis, which was 43%, and Cytobacillus kochii, which was 44.40%. The flocculating activity has the potential as a flocculating agent.

Keywords: *Bioflocculant activity, Shrimp ponds, Molecular identification.*

1. Introduction

The development of fish farming technology is a research topic that is urgently needed to increase fish farming production (Langdon, 2003)**.** In 2020, fishery production reached 214 million tons or IDR 424 billion. Aquatic biota production in 2020, 60% higher than the 1990 average, far exceeded world population growth, largely due to increased aquaculture production. Globally, aquaculture production provides about 17% of animal protein, reaching more than 50% in some countries in Asia and Africa. Nearly 7 billion demands for aquatic biota continues to increase. Therefore, the expansion and intensification of aquaculture production is urgently needed (FAO 2022). First, the aim of expanding aquaculture production is to produce a wider variety of aquaculture species without significantly increasing the excessive use of water and land resources (Avnimelech, 1999). The second goal is to develop sustainable aquaculture systems that do not damage the environment (Naylor *et al*., 2000). The third goal is to build a system that provides a socially and economically balanced cost/benefit balance (Avnimelech, 1999). Biofloc technology can meet all three of these requirements for sustainable aquaculture development by using bacteria as floc-forming agents. Providing vannamei shrimp production as a solution to the needs of the most promising aquaculture biota.

The production of vannamei shrimp is increasing due to technological advances. Currently, many vannamei shrimp farming systems are being developed with super-intensive systems. Application of effective technology for water treatment but there are challenges in intensive aquaculture operations. The high composition of the feed and faeces of shrimp that are not eaten in the culture container will produce organic matter which can be harmful to the shrimp. Fecal material and leftover feed that settles on the bottom of the sediment can interfere with the interaction of biota in the pond so that technology is needed to reduce this organic matter. Flocculation is an alternative method to address the problem of aquaculture wastewater which is a cheap, easy and effective technique for removing cell debris, colloids and suspended particles (Zhang *et al* 2012; Kurniawati *et al*., 2021). Compared to other conventional systems, this method is volume-independent for concentrating dead cells. This works with the help of flocculants which will change the nature of suspended particulate matter and allow it to form aggregates or small lumps (Newman, 2011). Many microorganisms including algae, bacteria and fungi isolated from sludge and sewage are reported to excrete extracellular polymeric substances such as functional proteins, exopolysaccharides, polysaccharides, glycoproteins, proteins, nucleic acids and cellulose. Therefore, to ensure the long-term sustainability of the vannamei shrimp cultivation industry, the flocculation method can be used as an alternative in the cultivation system.

Many studies have been conducted on the use of biofloc technology in sustainable cultivation systems with various types of bacteria. Previous studies have succeeded in isolating bacteria that produce high extracellular polymers as agents for forming biofloc systems such as functional proteins, exopolysaccharides, glycoproteins, nucleic acids, and cellulose (Feng and Xu, 2008). These bacteria were identified *as Bacillus licheniformis* (Xiong *et al*., 2010), *Peanebacillus* sp, *Bacillus* sp, and *Vagococcus* sp. The *Vagocoss* sp strain was identified as a bacterium that secreted the most extracellular polymers isolated from wastewater samples collected at Little Moon River Beijing (Gao *et al*., 2006). Other bacteria are *Bacillus firmus* (Salehizadeh and Shojaosadati, 2002), *Citrobacter* (Fujita *et al*., 2018), *Enterobacter aerogenes* (Lu *et al*., 2005), *Bacillus subtillis*, *Bacillus licheniformis, Pacilomyces, Nocardia amarae* (Deng *et al*., 2005), *Lactobacillus delbrukii* and *Bacillus alvei* (Abdel-Aziz *et al*., 2014). The isolation of bacteria has been studied extensively by researchers. However, some deficiencies or gaps must be addressed to improve the accuracy of the results and the bacteria are examined for bacteria that can potentially be used as biofloc candidates. In Indonesia, it is not clear what types of bacteria are used in biofloc systems for aquaculture. So, it is necessary to identify the types of bacteria that can be applied to biofloc system fish cultivation.

2. Materials And Methods

Sampling of bioflocculant-producing bacteria

Biofloculant bacterial isolate samples were taken from water and sediments of Pangandaran coastal shrimp ponds (5 ° 34'18.32'N, 102 ° 48'25.86'E) from January-April 2023. Bacterial isolate samples were taken using the purposive sampling method. Water and sediment samples were taken from two different ponds, namely the intensive pond and the traditional pond. Sampling points in intensive ponds are from the inlet water, outlet water, and sediment at the bottom of the pond, whereas the traditional pond sampling points are from the inlet water, middle water, outlet water, and sediment at the bottom of the pond.

Isolation of bioflocculant producing bacteria

The isolation of bacteria from water and sediment samples was conducted following the procedure of Nurhafid *et al*., (2021). A total of 0.5 ml of water samples and dissolved sediment samples were put into a test tube containing 4.5 ml of 0.9% NaCl as a 10^{-1} dilution and then homogenized using a vortex. A sample of 0.5 ml was then homogenized from the 10^{-1} dilution into the 10^{-2} dilution test tube. Do this step until the dilution tube is a 10^{-5} dilution. Furthermore, the results of the sample dilution were grown on marine agar media using the pour plate method (Rosiak *et al*., 2020) and incubated at 28°C for 48 hours. Bacteria that grow in single colonies were streaked for purification of selected bacteria based on colony morphology.

Bacterial sample purification

Bacterial purification was conducted using the streak plate method. Bacterial isolates selected based on different colony morphology were streaked on marine agar media and incubated for 24 hours at 28°C. The purified bacteria were streaked on marine agar in an inclined tube as stock for the bacterial flocculation test.

Bacterial flocculation activity test

A bacterial flocculation test was conducted on marine broth media which was added with 5% kaolin clay and $CaCl_2$ (flocculant medium). Bacterial samples were taken using an use needle from the stock and put into a test tube containing 5 ml of flocculant media. Bacterial samples were incubated on an incubator shaker at 150 rpm at 28°C for 70 hours. Bacterial flocculation was seen by looking at the flocs that form at the bottom of the test tube. Then, the results of bacterial incubation were measured using a spectrophotometer with a wavelength of 550 nm. Bacterial flocculation activity was measured using the following formula:

Flocculation activity = (B−A) B x 100% (Kurane & Matsuyama, 1994)

Where :

A = Sample absorbance value

B = Control absorbance value

Identification of Bioflocculant Bacteria based on 16s rDNA

Identification of bacteria was conducted based on the similarity of the 16S rDNA sequence with the data in GenBank. Shortly, bacterial gDNA was extracted using the PrestoTM Mini gDNA Bacteria Kit according to Geneaid's instructions to obtain pure DNA solutions. After obtaining pure DNA, the samples were PCR using the primers used in the amplification following the research by Palkova *et al*., (2021) 27f (5'- AGA GTT TGA TCC TGG CTC AG -3') and 1392r (5'- GGT TAC CTT GTT ACG ACT T -3') with an amplification result of 1500bp. The PCR program used for amplification was predenaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 20 seconds followed by final extension at 72°C for 5 minutes and storage at 25°C for 1 minute. Visualization of the PCR results using gel electrophoresis with an amplification product length of 1500bp. Then, the PCR results were sequenced and read for the 16s rDNA nucleotide bases. The complete sequence results were analyzed using the Basic Local Alignment Search Tool (BLAST) using an online program, namely NCBI (http://ncbi.nlm.nih.gov/) and phylogenetic analysis was performed using Molecular Evolutionary Genetic Analysis Software (MEGA11).

3. Results and Discussion

Flocculation activity

Based on testing the flocculation activity of 224 bacterial isolates from the seven points, five bacterial isolates had a relatively high value. These values can be seen in table 1.

Table 1. Bacterial flocculation activity

Isolate Code	OD	Concentration	Flocculation activity (%)
II12	550	2,429	21,70
II14	550	2,413	20,90
II26	550	2,487	24,60
IO25	550	2,439	43,00
TI25	550	2,380	44,40

Source: Authors

Molecular identification of flocculating bacteria

Based on the highest flocculation activity, 5 selected bacterial isolates were obtained, namely II14, II26, SI19, IO25, TI25. The selected isolates were identified molecularly based on the 16s rDNA gene. Identification was performed using 16s rDNA gene amplification with the help of a PCR

machine to obtain a 1500bp amplicon product. The results of the amplicon products were sequenced to obtain sequences for blast analysis on GenBank. Analysis of blast results is shown in Table 2.

Table 2. Blast analysis of 16s rDNA sequences from bacterial flocculating agents

Isolate Code	Blast Result	Query Cover (%)	*E. Value*	Identical (%)	Accession Number
II12	*Vibrio Navarensis* Strain 34	98	0,0	98.90	MT974084.1
II14	*Pseudoalteromonas ganghwensis* strain WAB2121	100	0,0	99.66	MH169282.1
II26	*Staphylococcus gallinarum* strain E2	99	0.0	98.30	MH371285.1
IO25	*Pseudoalteromonas ganghwensis* strain WAB2121	100	0.0	99.58	MH169282.1
TI25	*Cytobacillus kochii* strain FJAT-46230	100	0,0	90,89	MK859981.1

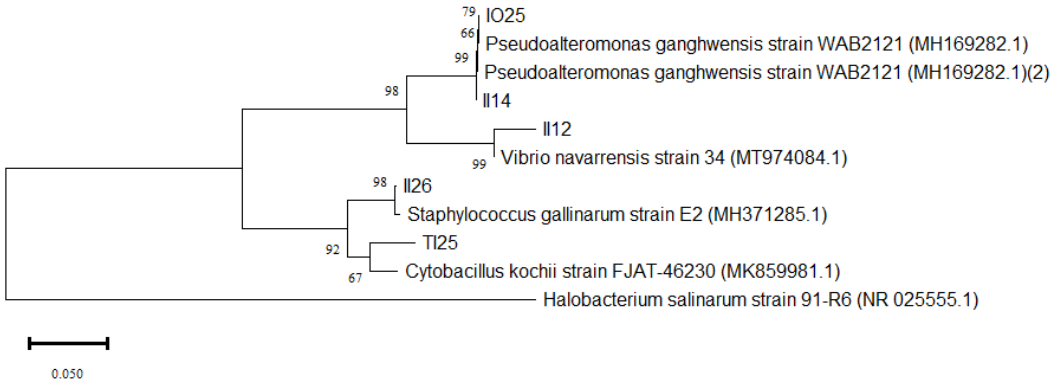

Figure 1. Bacterial phylogenetic tree of bioflocculant agents and closely related strains based on 16s rDNA sequences. NCBI access numbers are shown in brackets. Tree construction using evolutionary techniques is calculated based on Neighbor-joining and bootstrap values applied to 1000 replications. Bacterial strains that do not have an accession number are sample bacteria. Branching on Halobacterium salinarum strain 91-R6 (NR_025555.1) as out-group.

Vannamei shrimp cultivation with an intensive system in Indonesia has experienced a very high increase (Rangka & Gunarto, 2012 ; Amalisa *et al.*, 2021 ; Deriyanti *et al.*, 2021).. Shrimp cultivation with an intensive system is carried out by stocking large amounts of shrimp and providing high feed (Farras *et al*., 2017 ; Satyantini *et al.*, 2020), so these waters can contain a large amount of organic matter (Arifin *et al*., 2018 ; Ningrum *et al.*, 2019). Organic materials contained in intensive ponds are generally utilized by various types of degrading bacteria (Masithah *et al*., 2016 ; Jefri *et al*., 2020 ; Fitriadi *et al.*, 2023). Degrading bacteria in intensive ponds generally plays a role in improving the environment, one of which is floc-forming bacteria. Floc-forming bacteria or what is commonly called biofloc technology has been widely used in intensive ponds as a solution in dealing with water quality degradation and organic matter degradation (Rangka & Gunarto, 2012). Biofloc technology is an alternative technology that utilizes floc-producing bacteria to stabilize water quality, decompose organic matter, reduce ammonia, suppress pathogenic bacteria, and serve as food for animals (Maghfira, 2020).

In this study, 244 bacterial isolates from intensive ponds and traditional ponds in Pangandaran were tested for flocculation activity. According to the test, the flocculant activity obtained ranged from 20.9 to 44.40%. The range of these values is quite large but does not meet the requirements of a bioflocculant bacteria. According to Bukhari *et al*., (2015), bioflocculant bacteria have a flocculation activity of >40%. Meanwhile, according to Gao *et al*., (2006) and Abd-El-Haleem *et al*., (2008) good flocculation activity so that it can be said to be a bioflocculant bacteria is >75%. The high flocculation activity is characterized by an increase in kaolin solution saturation after the addition of bioflocculant

bacteria, accompanied by precipitate. The addition of CaCl2 in flocculation media acts as a source of cations. The cations are used as coagulants to neutralize the charge between colloidal particles so that they can combine to form flocs. During flocculation, a bond is formed between the bioflocculant and $CaCl_2$ that binds colloidal solids (kaolin) (Bakar *et al*., 2021).

The result of flocculating bacteria identification obtained 4 different species, namely *Vibrio Navarensis* Strain 34, *Pseudoalteromonas ganghwensis* strain WAB2121, *Staphylococcus gallinarum* strain E2, and *Cytobacillus kochii* strain FJAT-46231. Some of the bacteria obtained in this study have been found to produce quite high flocculation activity in previous studies. According to Fu *et al*., (2021), the bacteria *Pseudoalteromonas* sp. has a flocculation activity of 94.5% in an incubation time of 72 hours and 78% in an incubation time of 48 hours (Harun *et al.*, 2018). *Pseudoalteromonas* sp. able to flocculate various dissolved solids such as kaolin clay, activated carbon, soil, magnesium oxide and aluminum oxide. This ability makes the bacterium *Pseudoalteromonas* sp. can be used as an effective flocculant for wastewater treatment at low temperatures and salinity (Li *et al*., 2008). Bacteria *Staphylococcus* sp. able to produce bioflocculant with high flocculation activity, namely 70.3% for kaolin suspension. Bacteria *Staphylococcus* sp. has potential as a substitute for conventional synthetic flocculants (Wong *et al*., 2012). According to this study, *Cytobacillus kochii* bacteria had the highest flocculation activity of 44.40%, which is quite high as a flocculating agent. Bukhari *et al*., (2015) stated that bacteria with a flocculation activity value of >40% had potential as a flocculating agent.

The flocculation activity value of the bacteria identified in this study did not reach >60%, influenced by various factors such as pH, temperature, and bacterial suspension. According to Bakar *et al*., (2021), the flocculation activity of bacteria is influenced by molecular weight, pH, temperature, and metal ions. The pH value of the solution determines the flocculation activity and affects the stability of suspended particles and floc formation. According to Tawila *et al*., (2018) very acidic conditions (pH value < 5) and very alkaline conditions (pH value > 9) reduced the flocculation activity (< 70%) of bioflocculant produced by *Bacillus salmalaya*. Factors that influence the flocculation activity of biofloculant bacteria are carbon sources, nitrogen sources, carbon and nitrogen ratios, temperature, pH, dissolved oxygen, and culture time (Sofie *et al*., 2017). Some bacteria require stimulation of metal ions to produce flocculants (Razali *et al*., 2011).

There are several interactions involved in the formation of flocculants, including biological interactions and chemical interactions, as has been demonstrated in several previous studies. Although there are many components that form flocculants such as microalgae (Nasir et al., 2019), this research attempts to focus on studying groups of enzyme-producing bacterial microorganisms that can bind all the components of the buffer to form floc clumps. This research is the basis for obtaining bacterial strains that have an important role in the process of flocculant formation by manipulating growth medium enriched with kaolin to stimulate flocculant forming enzymes. This approach has been extensively studied in detecting enzyme-producing bacteria in flocculant formation (Araújo et al., 2018; Bahniuk et al., 2022; M. Kumar et al., 2019). Then the results of this study showed low flocculant activity compared to the study of Bukhari et al., (2015) but several isolates in this study had a percentage of flocculant activity that was included in the percentage of flocculant activity found by Kurniawan et al., (2021) namely <30% -72 .5%. The majority of the bacteria identified in this study were new strains showing flocculant activity, especially in vannamei shrimp ponds. There is a big opportunity here, especially when it comes to the cultivation of *vannamei shrimp* using biotechnology.

Flocculant forming bacteria have a very important role in aquaculture. Besides the utilization of organic matter, which can become toxic, flocculant bacteria use degradation products to form floc clumps, which can provide natural food for cultivated organisms. This principle opens up great opportunities for farmers and researchers, especially in the study of water quality and natural feed in aquaculture using bacterial biotechnology. In previous studies, the *Pseudoalteromonas ganghwensis* strain is a bacterium as a probiotic candidate that has the ability to produce protease enzymes and can suppress the growth of *Vibrio parahaemolyticus* which is the main pathogen of *vannamei shrimp* culture (Fitriadi et al., 2023). In the future, floc-forming bacteria can be packaged as probiotic products that can be utilized on a field scale or hatchery scale cultivation which can minimize land

and biocontrol pathogens. In the present day, several countries around the world are developing this technology as the biofloc cultivation system.

4. Conclusion

In this study, the results showed a flocculation activity of the five isolates rangeing from 20.9-to 44.40%. In this study, the bacteria *Pseudoalteromonas ganghwensis* recorded the highest flocculant activity at 43%, while *Cytobacillus kochii* at 44.40%. The flocculating activity has the potential as a flocculating agent that needs to be reviewed before being used as a bacterial biofloculant.This research is basic research regarding the detection of flocculant-forming bacteria, this research has limited data as well as a complex and detailed study of how bacteria bind various flocculant-forming components. Aquaculture productivity could be increased in the future by increasing flocculant-forming bacteria, which can be used as biotechnology products such as probiotics.

Acknowledgements:
The authors would like to thank Jenderal Soedirman University, Banyumas, Indonesia, for providing Research Program Grants for applied research in the Fiscal Year of 2023 (UNSOED Grants) as financial support for this research.

Conflict of interest:
The authors declare no conflict of interest.

References:

Abdel-Aziz, S. M., Hamed, H. A., Keera, A. A. 2014. Cold-Shock Stress Induces Formation of a Biopolymer Flocculant by *Bacillus alvei* NRC-14 under Static Incubation Conditions. *Scholarena Journal of Biotechnology*, 1(2): 1–9. https://doi.org/10.18875/2375-6713.1.201

Abd-El-Haleem, D. A. M., Al-Thani, R. F., Al-Mokemy, T., Al-Marii, S., Hassan, F. 2008. Isolation and Characterization of Extracellular Bioflocculants Produced by Bacteria Isolated from Qatari Ecosystems. *Polish Journal of Microbiology*, 57(3): 231-239.

Abu Bakar, S. N. H., Abu Hasan, H., Abdullah, S. R. S., Kasan, N. A., Muhamad, M. H., Kurniawan, S. B. 2021. A review of the production process of bacteria-based polymeric flocculants. *Journal of Water Process Engineering*, 40(1): 101915. https://doi.org/10.1016/j.jwpe.2021.101915

Amalisa., Mahasri, G., Kismiyati. 2021. The correlation between ectoparasite infestation and total *Vibrio parahaemolyticus* bacteria in Pacific white shrimp (*Litopenaeus vannamei*) in Super Intensive Ponds. *In IOP Conf. Series: Earth and Environmental Science,* 888 (2021): 1-8. https://doi.org/10.1088/1755-1315/888/1/012003.

Araújo, D., Concórdio-Reis, P., Marques, A. C., Sevrin, C., Grandfils, C., Alves, V. D., Fortunato, E., Reis, M. A. M., and Freitas, F. 2018. Demonstration of the ability of the bacterial polysaccharide FucoPol to flocculate kaolin suspensions. *Environmental technology*. 1-28. https://doi.org/10.1080/09593330.2018.1497710.

Arifin, N. B., Fakhri, M., Yuniarti, A., Hariati, A. M. 2018. Phytoplankton Community in the Vaname Shrimp Intensive Cultivation System, *Litopenaeus vannamei* in Probolinggo, East Java. *Jurnal Ilmiah Perikanan Dan Kelautan*, 10(1): 46–53. *https://doi.org/10.20473/jipk.v10i1.8542*.

Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176(3–4): 227–235. https://doi.org/10.1016/S0044-8486(99)00085-X

Bahniuk, M. S., Alidina, F., Tan, X., Unsworth, L. D. 2022. The last 25 years of research on bioflocculants for kaolin flocculation with recent trends and technical challenges for the future. *Frontiers in Bioengineering and Biotechnology*, 10: 1-24 https://doi.org/10.3389%2Ffbioe.2022.1048755

Bukhari, N. A., Bakar, N. A., Loh, S. K., Zairun, M. A. 2015. Isolation and identification of novel bioflocculant-producing bacteria from palm oil mill effluent. *Journal of Pure and Applied Microbiology*, 9(1): 1–12.

Deng, S., Yu, G., and Ting, Y. P. 2005. Production of a bioflocculant by *Aspergillus parasiticus* and its application in dye removal. *Colloids and Surfaces B: Biointerfaces*, 44(4): 179–186. https://doi.org/10.1016/j.colsurfb.2005.06.011

Deriyanti, A., Mahasri, G., Kismiyati, K. 2021. Ectoparasite infestation and *Vibrio alginolyticus* bacterial infection in super-intensive ponds with high ammonia levels of Penaeus vannamei. In *E3S Web of Conferences*. 322(2021): 1-8. EDP Sciences. https://doi.org/10.1051/e3sconf/202132202002.

Farras, A., Mahasri, G., Suprapto, H. 2017. Prevalence and Degrees of Infestation Ectoparasite on White Shrimp (*Litopenaeus vannamei*) in Intensive an. *Jurnal Ilmiah Perikanan dan Kelautan*, 9(2): 118. https://doi.org/10.20473/jipk.v9i2.7640.

Feng, D. L., and Xu, S. H. 2008. Characterization of bioflocculant MBF3-3 produced by an isolated *Bacillus* sp. *World Journal of Microbiology and Biotechnology*, 24(9): 1627–1632. https://doi.org/10.1007/s11274-008-9654-1

Fitriadi, R., Setyawan, A. C., Palupi, M., Nurhafid, M., Kusuma, R. O. 2023. Isolation and molecular identification of proteolytic bacteria from vaname shrimp (*Lithopenaeus Vannamei*) ponds as probiotic agents. *Iraqi Journal of Veterinary Sciences*, *37*(1): 161-170. https:// 10.33899/ijvs.2022.133468.2251

Fu, W., Liao, M., Zhang, D., Zhou, Y., Yang, H. 2021. Studies on Bioflocculant Production by *Pseudoalteromonas* sp. NUM8, a Marine Bacteria Isolated from the Circulating Seawater. *Journal of Ocean University of China*, 20(5): 1276–1284. https://doi.org/10.1007/s11802-021-4747-7

Fujita, M., Ike, M., Jang, J. H., Kim, S. M., Hirao, T. 2018. Bioflocculation production from lower-molecular fatty acids as a novel strategy for utilization of sludge digestion liquor. *Water Science and Technology*, 44(10): 237–243. https://doi.org/10.2166/wst.2001.0630

Gao, J., Bao, H. ying, Xin, M. xiu, Liu, Y. xia, Li, Q., Zhang, Y. fen. 2006. Characterization of a bioflocculant from a newly isolated *Vagococcus* sp. W31. *Journal of Zhejiang University. Science. B.*, 7(3): 186–192. https://doi.org/10.1631/jzus.2006.B0186

Harun, A. A. C., Ghazali, N. A., Hashim, N. F. C., Mohammad, N. A. H., Ikhwanuddin, M., Ismail, N., Ibrahim, Z., Kasan, N. A. 2018. The potential of bioflocculant- producing bacteria as inoculum for biofloc based systems. *Journal of Environmental Biology*, 39(9): 917–922.

Jefri, M., Satyantini, W. H., Sahidu, A. M., Nindarwi, D. D. 2020. Application of Probiotics for Organic Matter and Enhancement of Growth Performance in White Shrimp (*Litopenaeus vannamei*). *Jurnal Ilmiah Perikanan Dan Kelautan*, *12*(1): 97-104. https://10.20473/jipk.v12i1.16618

Kumar, N., Banerjee, C., Kumar, N., Jagadevan, S. 2019. A novel non-starch based cationic polymer as flocculant for harvesting microalgae. *Bioresource technology*, 271: 383-390. https://doi.org/10.1016/j.biortech.2018.09.073

Kurane, R., and Matsuyama, H. 1994. Production of a Bioflocculant by Mixed Culture. *Bioscience, Biotechnology, and Biochemistry*, 58(9): 1589–1594. https://doi.org/10.1271/bbb.58.1589

Kurniawan, S. B., Abdullah, S. R. S., Othman, A. R., Purwanti, I. F., Imron, M. F., Ahmad, A., Hasan, H. A. 2021. Isolation and characterisation of bioflocculant-producing bacteria from aquaculture effluent and its performance in treating high turbid water. *Journal of Water Process Engineering*, 42: 1-10. https://doi.org/10.1016/j.jwpe.2021.102194

Kurniawati, F. A., Masithah, E. D., Rahardja, B. S. 2021. The Effect of Commercial Probiotics on the Phytoplankton Diversity Associated with Biofloc. *World's Veterinary Journal*, *11*(4), 725-730. https://dx.doi.org/10.54203/scil.2021.wvj92

Langdon, C. 2003. Microparticle types for delivering nutrients to marine fish larvae. *Aquaculture*, 227(1–4): 259–275. https://doi.org/10.1016/S0044-8486(03)00508-8

Li, W. W., Zhou, W. Z., Zhang, Y. Z., Wang, J., Zhu, X. B. 2008. Flocculation behavior and mechanism of an exopolysaccharide from the deep-sea psychrophilic bacterium *Pseudoalteromonas* sp. SM9913. *Bioresource Technology*, 99(15): 6893–6899. https://doi.org/10.1016/j.biortech.2008.01.050

Lu, W. Y., Zhang, T., Zhang, D. Y., Li, C. H., Wen, J. P., Du, L. X. 2005. A novel bioflocculant produced by *Enterobacter aerogenes* and its use in defecating the trona suspension. *Biochemical Engineering Journal*, 27(1): 1–7. https://doi.org/10.1016/j.bej.2005.04.026

Maghfira. 2020. Characterization of Biofloc-forming Flocculation Bacteria in Tilapia (*Oreochromis niloticus*) Ponds in Pangkep Regency. *CELEBES BIODIVERSITAS : Jurnal Sains dan Pendidikan Biologi*, *3*(2):1-7. https://doi.org/10.51336/cb.v3i2.209

Masithah, E. D., Octaviana, Y. D., Manan, A. 2016. The Effect of Differences in Commercial Probiotics on the C:N and N:P Ratios of Biofloc Culture Media in Experimental Tubs. *Journal of Aquaculture and Fish Health*, 5(3): 118–125. https://doi.org/10.20473/jafh.v5i3.11333.

Nasir, N. M., Yunos, F. H. M., Jusoh, H. H. W., Mohammad, A., Lam, S. S., Jusoh, A. 2019. Subtopic: Advances in water and wastewater treatment harvesting of Chlorella sp. microalgae using *Aspergillus niger* as bio-flocculant for aquaculture wastewater treatment. *Journal of environmental management*, 249(2019):1-7. https://doi.org/10.1016/j.jenvman.2019.109373

Naylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M. C. M., Clay, J., Folke, C. I. J. L., Mooney, H., Troell, M. 2000. Effect of aquaculture on world sh supplies. *Nature*, 409: 1017–1024. https://doi.org/10.1038/35016500

Newman S.G., 2011 Understanding Bioflocculation in Aquaculture Producing System. AQUA CultureAsia Pacific Magazine March/April, 25-27, Retrieved from http://www.aquaculturewithoutfrontiers.org

Ningrum, D., Arief, M., Pursetyo, D. K. 2019. Comparative test on bacteria in the digestive tract of vannamei shrimp (*Litopenaeus vannamei*) at intensive and extensive ponds in Ujungpangkah, Gresik. *In IOP Conf. Series: Earth and Environmental Science*, 236 (2019):1-11. https://10.1088/1755-1315/236/1/012030.

Nurhafid, M., Syakuri, H., Oedjijono, O., Listiowati, E., Ekasanti, A., Nugrayani, D., & Pramono, H. 2021. Isolation and Molecular Identification of Proteolytic Bacteria from the Digestive Tract of Tilapia (*Oreochromis niloticus*) Cultured in Banyumas Regency. *Jurnal Perikanan Universitas Gadjah Mada*, 23(2): 95. https://doi.org/10.22146/jfs.64072

Palkova, L., Tomova, A., Repiska, G., Babinska, K., Bokor, B., Mikula, I., Minarik, G., Ostatnikova, D., Soltys, K. 2021. Evaluation of 16S rRNA primer sets for characterisation of microbiota in paediatric patients with autism spectrum disorder. *Scientific Reports*, 11(1): 1–13. https://doi.org/10.1038/s41598-021-86378-w

Rangka, N. A., and Gunarto. 2012. Effect of Biofloc Growth on Intensive Vaname Shrimp Cultivation in Ponds. *Jurnal Imiah Perikanan Dan Kelautan*, *4*(2): 141–149. *https://doi.org/10.20473/jipk.v4i2.11565*.

Razali, M. A. A., Ahmad, Z., Ahmad, M. S. B., Ariffin, A. 2011. Treatment of pulp and paper mill wastewater with various molecular weight of polyDADMAC induced flocculation. *Chemical Engineering Journal*, 166(2): 529–535. https://doi.org/10.1016/j.cej.2010.11.011

Rosiak, E., Madras-Majewska, B., Gemba, M. 2020. Selected Methods Of Microbiological Quality Assessment Of Lime And Acacia Honeys Available On Polish Market. *Nauka Technologia Jakość*, 27(1): 85 – 95.

Salehizadeh, H., & Shojaosadati, S. A. 2002. Isolation and characterisation of a bioflocculant produced by *Bacillus firmus. Biotechnology Letters*, 24(1): 35–40. https://doi.org/10.1023/A:1013853115624

Satyantini, W. H., Salsabila, M., Nindarwi, D. D., Sahidu, A. M., Mukti, A. T. 2020. Provision of bacteria from shrimp pond sediment towards N/P ratio, plankton abundance, and total bacteria in the culture media of white shrimp (*Litopenaeus vannamei*). *In IOP Conf. Series: Earth and Environmental Science*, 441(2020): 1-8. https://10.1088/1755-1315/441/1/012139.

Sofie, V. D. H., Rodrigues, A., Hamaekers, H., Sonnenholzner, S., Vervaeren, H., Boon, N. 2017. Microalgal bacterial flocs treating paper mill effluent: A sunlight-based approach for removing carbon, nitrogen, phosphorus, and calcium. *New Biotechnology*, 39: 1–10. https://doi.org/10.1016/j.nbt.2017.03.004

Tawila, Z. M. A., Ismail, S., Dadrasnia, A., Usman, M. M. 2018. Production and characterization of a bioflocculant produced by *bacillus salmalaya* 139si-7 and its applications in wastewater treatment. *Molecules*, 23(10). https://doi.org/10.3390/molecules23102689

Wong, Y. S., Ong, S. A., Teng, T. T., Aminah, L. N., Kumaran, K. 2012. Production of bioflocculant by *staphylococcus cohnii* ssp. from Palm Oil Mill Effluent (POME). *Water, Air, and Soil Pollution*, 223(7): 3775–3781. https://doi.org/10.1007/s11270-012-1147-z

Xiong, Y., Wang, Y., Yu, Y., Li, Q., Wang, H., Chen, R., He, N. 2010. Production and characterization of a novel bioflocculant from *Bacillus Licheniformis*. *Applied and Environmental Microbiology*, 76(9): 2778–2782. https://doi.org/10.1128/AEM.02558-09

Zhang, C., Cui, Y., Wang, Y. 2012. Bioflocculant produced from bacteria for decolorization, Cr removal and swine wastewater application. *Sustain. Environ. Res.*, 22(2): 129-134.