

Molecular identification of bacteria isolated from culture medium of rotifer fed on fishery waste diet

by Elvy Like Ginting 22

Submission date: 01-Mar-2021 04:04PM (UTC-0800)

Submission ID: 1521752993

File name: ted_from_culture_medium_of_rotifer_fed_on_fishery_waste_diet.pdf (418.29K)

Word count: 5048

Character count: 27013

Molecular identification of bacteria isolated from culture medium of rotifer fed on fishery waste diet

STENLY WULLUR^{1*}, HATOPAN NAPITUPULU¹, LETHA LOISE WANTANIA¹, ELVY LIKE GINTING¹, VEIBE WAROUW¹, SANDRA TILAAAR², TRINA EKAWATI TALLEI², INNEKE FENNY MELKE RUMENGAN¹

¹Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus, Bahu, Malalayang, Manado 95115, North Sulawesi, Indonesia.

Tel./fax.: +62-431-868027, *email: stenlywullur@unsrat.ac.id

²Faculty of Mathematics and Science, Sam Ratulangi University, Jl. Kampus Unsrat, Manado 95115, North Sulawesi, Indonesia.

Manuscript received: 5 April 2020. Revision accepted: 24 May 2020.

Abstract. Wullur S, Napitupulu H, Wantania LL, Ginting EL, Warouw V, Tilaar S, Tallei TE, Rumengan IFM. 2020. Molecular identification of bacteria isolated from culture medium of rotifer fed on fishery waste diet. *Biodiversitas* 21: 1735-1740. The aim of this study was 16S-rRNA sequences based molecular identification of bacteria isolated from culture medium of rotifer fed with fishery waste diet (FWD). We cultured rotifer *Brachionus rotundiformis* in sterilized seawater (salinity 25 ppt) using FWD, following the procedure in Patent No. P00201609066. Bacteria from the culture were collected, homogenized, diluted 10 to 1000 fold, spread on agar plates and incubated at 37°C for 24 to 48 hours. Representative colonies of the bacteria according to their morphologies were isolated for further characterization. Genomic DNA of the isolates were extracted, and the 16S rRNA gene of the isolates were amplified. Polymerase Chain Reaction (PCR) product of each isolate was sequenced and queried against the NCBI GenBank database. Six different isolates based on size, color, elevation, margin, and colony were observed during 24-48 hours incubation at 37°C. The 16S rRNA genes of the six isolates were successfully amplified and produced DNA band at 1300-1500 bp, with quality value equal to or greater than 20 (QV20+) of each entire sequence around 941-1253 bases. Basic Local Alignment Search Tool (BLAST) queries in the NCBI GenBank and EzBioCloud database using the 16S-rRNA gene sequences showed that the six isolates belong to four different genera, i.e: *Bacillus*, *Staphylococcus*, *Vibrio*, and *Alteromonas*.

Keywords: Bacteria, diet, fish-waste, identification, rotifer, 16S rRNA

25 INTRODUCTION

The use of rotifer (*Brachionus plicatilis* species complex) as live-food for newly hatched fish larvae was initiated in early 1960s (Hagiwara et al. 2014; Hagiwara et al. 2017). Today, most hatcheries use the rotifers as indispensable live-food for fish larviculture (Kotani et al. 2017; Wullur 2017) due to their benefits such small body size, rapid reproductive rate, high population growth, relatively slow in motility, mass culture possible and its nutritional value (Wullur et al. 2011; Wullur et al. 2013; Hagiwara et al. 2014; Rumengan et al. 2016; Wati and Imanto 2018; Wullur et al. 2018; Lee et al. 2019).

Procedure used today for culturing the rotifers uses microalgae i.e. *Chlorella* sp. *Nannochloropsis oculata*, etc (Hagiwara et al. 2017). In fact, production cost of microalgae is high due to the high investment costs and running expenses (Acien et al. 2017). For this reason, cheap food sources based on baker's yeast were introduced (Hagiwara et al. 2014; Hagiwara et al. 2017), but it provided less nutritional value particularly for eicosapentaenoic acid (20:5 n-3) and docosahexaenoic (22:6 n-3) to support growth of fish larvae. Also, the baker's yeast often a source of unstable culture or even culture crashes due to the rapid decline of water quality of the culture and therefore is currently less attractive for fish farmers (Hagiwara et al. 2014; Hagiwara et al. 2017; Ogello et al. 2018; Ogello et al. 2019; Wullur et al. 2019;

18 Ogello et al. 2020). During the late 1980s, a Japanese *Chlorella* industry company (Fukuoka, Japan) has developed a microalga paste *Chlorella V-12*® as food for rotifer. The microalgal paste is convenient since it is ready-to-use and could be stored for about 2 weeks without significant loss of its nutritional value (Hagiwara et al. 2014; Hagiwara et al. 2017). The microalgal paste is also more stable food for rotifer that enables aquaculturists to mass culture rotifer at high density but its price is high and not commonly available in hatcheries particularly in developing countries (Hagiwara et al. 2014). Recently, a cheap and ready-to-use diet for rotifer based on fishery-waste is being developed as an alternative to the microalgae-based feed (Ogello et al. 2018; Napitupulu et al. 2019; Ogello et al. 2019; Wullur et al. 2019; Ogello et al. 2020). Acceptance of rotifer on the diet was great, where population growth of rotifer fed the diet increased significantly about 2000 to 3000 ind./ml within approximately 3 to 5 days culture (Wullur et al. 2017), and its dietary value to fish larvae was comparable to rotifer fed with microalgae paste (Ogello et al. 2018; Ogello et al. 2019; Wullur et al. 2019; Ogello et al. 2020).

The fishery diet acts as a source for bacterial bloom which further used by the rotifer as their nutritional source for growth and development, but the bacterial species involved in decomposing the fishery diet is unknown until recently. To address this lack of information, a small-ribosomal subunit 16S rRNA gene was used to identify

species of bacteria at molecular level in the present study. The 16S rRNA gene is commonly used to study bacterial taxonomy as it is present in almost all bacteria species, its function over time has not changed, and its length (1500 bp) is large enough for informatics purpose (Fuks et al. 2018; Wantania et al. 2019; Wondal et al. 2019; Akihary et al. 2020). Understanding the bacterial composition in rotifer cultures is important for the aquaculture industry.

42 MATERIALS AND METHODS

Procedures

Bacterial isolation and culture

Bacterial isolates in this study were obtained from culture of rotifer *Brachionus rotundiformis* fed with fishery waste diet. The procedure for the preparation of the fishery waste diet was adapted as described in Patent No. P00201609066 registered in Indonesia. Culture of rotifer was conducted using sterilized seawater at salinity 25 ppt and placed in a room at ambient temperature at around 25-28°C. Bacterial isolation was conducted by taking 1 mL water sample from the rotifer culture (rotifer population with the density of about 100 ind./ml) and was serially diluted (for 10 to 1000-fold) in sterile seawater, plated on 2% nutrient agar (NA). The NA plates were incubated for 24-48 hours at 37°C for bacterial colonies observations following Laboffe (2012). Representative distinct colonies of the bacteria according to their phenotype characters were isolated and grown in nutrient broth for 24-48 hours, harvested, and centrifuged (14,000 rpm for 5 minutes) for molecular analysis.

Molecular identification

Genomic DNA of the bacterial isolates were extracted using Qiaprep Miniprep Kit (Qiagen, Hilden, Germany) according to the manufacture's instruction. The bacterial 16S rRNA gene was amplified using the universal PCR primer pairs (Integrated DNA Technologies-IDT, Singapore) 8F (5'-AGAGTTTGATCCTGGCTCA-3') and 1492 R (5'-ACCTTGTTACGACTT-3') (Fuks et al. 2018; Wantania et al. 2019; Wondal et al. 2019; Akihary et al. 2020). The amplification reaction was performed in a total volume of 25 µl, consisting of 5 µl of 5x Hotfirepool, 17 µl of ddH₂O, 1 µl of Primer 8F, 1 µl of Primer 1492R, and 1 µl sample. Amplification for 35 cycles was performed in Professional Thermocycler (Biometra Analytik Jena). The temperature profile for PCR was 95°C for 6 min (1 cycle), 95°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec (35 cycles), followed by 72°C for 10 min at the end for final cycle. PCR products were electrophoresed on 1% agarose gel then visualized with ethidium bromide staining to check the success of PCR amplification. For DNA sequencing, the amplicon and the primers (forward and reverse) were sent to First-Base Co., Selangor, Malaysia. A BigDye® Terminator v.3.1 Cycles Sequencing Kit (Applied Biosystems, USA) was used bidirectionally sequence the DNA, and read using an ABI 377 automatic DNA sequencer. Sequences quality was assessed using Sequence Scanner version 2.0

Software (Applied Biosystem) and the sequence traces were trimmed, assembled and manually edited using Geneious Prime version 2020 (<http://www.geneious.com>, Kearse et al., 2012) prior to being submitted to 16S rRNA sequence BLAST analysis at The National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov/>) and EzBioCloud (<https://www.ezbiocloud.net/>) (Yoon et al. 2017). BLAST result data were limited to identity percent values between 97-100%, and query cover between 98-100%.

RESULTS AND DISCUSSION

It is well known that rotifers consume bacteria as their nutritional source for growth and reproduction (Wullur et al. 2019). In nature, the bacteria are important food components in rotifer microbial web, while in culture, the bacteria proved to have beneficial effect on culture quality of rotifers (Loo et al. 2016) and reared fish larvae (Nevejan et al. 2018). On the other hand, there are reports which indicated bacteria as possible cause of culture crash on rotifer or larval mortality due to pathogenic outbreak (Hache and Plante 2011). Population densities of rotifer cultured using the FWD in the present study were around 100 ind./mL at which the bacterial samples were collected. The capability of rotifer to grow and reproduce in FWD without any microalgae addition as the current procedure larviculture industry was also reported previously (Ogello et al. 2018; Ogello et al. 2019; Wullur et al. 2019; Ogello et al. 2020). We suggested that the FWD was the source of bacteria bloom inside the culture medium and further utilized by the rotifer as their nutritional source.

The size, color, elevation, margin, and type are well-known characters in characterization of bacterial colonies (Sousa et al. 2015). By collecting samples from the culture medium of rotifer, we successfully isolated 6 different colonies of bacteria based on the characters of colony morphology. All isolates in this study showed small sized round type colonies (isolates F0032, F0033, F0034, F0035, F0036, and F0037) except isolate F0031, having large-sized colony in comparison to other isolates. The colony color of isolates F0031, F0032, F0033, and F0035 were milky white, while whitish-pink for isolates F0036 and F0037. The colonies elevation was umbonate (F0031) and convex (F0032, F0035, F0036 and F0037), while the margins were erose (F0031) and smooth (F0032, F0033, F0035, F0036 and F0037).

Bacteria can be transmitted to fish or crustacean larvae when rotifers are given as food, and the bacteria could increase growth and survival of the larvae or vice versa. According to Fialkowska et al. (2019) and Onianwah et al. (2018), the main genera identified in rotifer and fish larviculture were *Pseudomonas*, *Vibrio*, *Moraxella*, and *Flavobacterium*. In the present study, based on BLAST result using database of 16S rRNA at GenBank and EzBioCloud gene marker, we identified 5 different bacterial species (*Bacillus cereus* species complex, *Vibrio rotiferanus*, *Bacillus jeotgali*, *Staphylococcus warneri*, and *Aleromonas fortis*) belonging to 4 different genera

(*Bacillus*, *Vibrio*, *Staphylococcus*, and *Alteromonas*) which present in the culture of rotifer fed FWD (Table 1).

Two isolates (F0031 and F0033) identified were belonged to genera of *Bacillus*, a genus of gram-positive, rod-shaped bacteria, that have been used for years as probiotics, which confer health benefits to host. The isolate F0031 has similar value (i.e. score, query cover, e-value and identity; 2593, 99%, 0.0, and 99.79%, respectively) to *B. wiedmannii* (NR_152692.1), *B. proteolyticus* (NR_157735.1) and *B. cereus* (NR_074540.1; 37_113266.1; NR_115714.1) (Table 1). According to Miller et al. (2016; 2018) and Liu et al. (2017), *B. wiedmannii*, *B. proteolyticus* and *B. cereus* were members of *B. cereus* species complex, which comprises about 12 closely related species, and other 9 new members of the *Bacillus* species complex as proposed by Liu et al. (2017). *B. cereus* is a gram-positive, aerobic-to-facultative, spore-

forming rod-shaped bacteria having wide distribution in the environment (Bottone 2010). *B. cereus* is a pathogenic bacterium that is harmful to humans and causes foodborne illness, but some strains of *B. cereus* have long been known as probiotic bacteria in rotifer culture (Md et al. 2015; Loka et al. 2016). In spite of that, *B. cereus* was also reported to have a significant role in enhancing growth performance of fish fingerlings (Chandran et al. 2014; Vargas-Albores et al. 2017; Sultani et al. 2019). Vargas-Albores et al. (2017) reported that *B. cereus* isolated from tiger shrimp (*Penaeus monodon*) and Pacific white shrimp (*Litopenaeus vannamei*) revealed growth and immune enhancer features for shellfish. *B. cereus* was reported to have the capability of enhancing various immune parameters, i.e. phenoloxidase, lysozyme, respiratory burst, and bactericidal activity in *P. monodon* (Chandran et al. 2014).

Table 1. Top five hits against Nucleotide BLASTed of 16S-rRNA gene of the bacterial isolates using rRNA type strains/prokaryotic16S_ribosomal RNA (bacteria and archaea) database setting in the NCBI GenBank

Isolates	Species & Accession number	Score	Query cover	E-value	Identity
F0031	<i>Bacillus wiedmannii</i> (NR_152692.1)	2593	99%	0.0	99.79%
	<i>Bacillus proteolyticus</i> (NR_157735.1)	2593	99%	0.0	99.79%
	<i>Bacillus cereus</i> (NR_074540.1)	2593	99%	0.0	99.79%
	<i>Bacillus cereus</i> (NR_113266.1)	2593	99%	0.0	99.79%
	<i>Bacillus cereus</i> (NR_115714.1)	2593	99%	0.0	99.79%
F0032	<i>Vibrio rotiferanus</i> (NR_118091.1)	2597	100%	0.0	99.65%
	<i>Vibrio rotiferanus</i> (NR_042081.1)	2597	100%	0.0	99.65%
	<i>Vibrio campbellii</i> (NR_119050.1)	2588	99%	0.0	99.58%
	<i>Vibrio campbellii</i> (NR_113782.1)	2577	100%	0.0	99.30%
	<i>Vibrio alginolyticus</i> (NR_118258.1)	2562	100%	0.0	99.23%
F0033	<i>Bacillus jeotgali</i> (NR_025060.1)	2567	99%	0.0	99.30%
	<i>Bacillus boroniphilus</i> (NR_041275.1)	2564	99%	0.0	99.23%
	<i>Bacillus thioarans</i> (NR_043762.1)	2562	99%	0.0	99.23%
	<i>Bacillus subterraneus</i> (NR_104749.1)	2551	99%	0.0	99.09%
	<i>Bacillus selenatarsenatis</i> (NR_041465.1)	2536	99%	0.0	99.22%
F0035	<i>Staphylococcus warneri</i> (NR_025922.1)	2603	100%	0.0	99.65%
	<i>Staphylococcus pasteurii</i> (NR_024669.1)	2579	100%	0.0	99.37%
	<i>Staphylococcus pasteurii</i> (NR_114435.1)	2562	99%	0.0	99.29%
	<i>Staphylococcus epidermidis</i> (NR_036904.1)	2516	100%	0.0	98.59%
	<i>Staphylococcus epidermidis</i> (NR_113957.1)	2512	100%	0.0	98.52%
F0036	<i>Alteromonas fortis</i> (MK007076) (EzBioCloud)	-	-	-	99.56%
	<i>Alteromonas litorea</i> (AY428573) (EzBioCloud)	-	-	-	99.34%
	<i>Alteromonas tagae</i> (NR_043977.2)	2407	100%	0.0	97.88%
	<i>Alteromonas litorea</i> (NR_025780.1)	2403	100%	0.0	97.88%
	<i>Alteromonas confuentis</i> (NR_137375.1)	2396	100%	0.0	97.71%
	<i>Alteromonas mediterranea</i> (NR_148755.1)	2390	100%	0.0	97.64%
F0037	<i>Alteromonas mediterranea</i> (NR_148754.1)	2388	100%	0.0	97.64%
	<i>Alteromonas fortis</i> (MK007076) (EzBioCloud)	-	-	-	99.40%
	<i>Alteromonas macleodii</i> (CP003841) (EzBioCloud)	-	-	-	99.16%
	<i>Alteromonas macleodii</i> (NR_114053.1)	2435	97%	0.0	98.68%
	<i>Alteromonas macleodii</i> (NR_037127.1)	2429	97%	0.0	98.76%
	<i>Alteromonas mediterranea</i> (NR_148755.1)	2422	100%	0.0	97.87%
	<i>Alteromonas litorea</i> (NR_025780.1)	2407	100%	0.0	97.65%
	<i>Alteromonas mediterranea</i> (NR_148753.1)	2405	100%	0.0	97.65%

Findings by He et al. (2017) suggested that the use of *B. cereus* in combination with *Clostridium butyricum* and *Lactobacillus acidophilus* can be superior to the growth and production of digestive enzymes of hybrid grouper (*Epinephelus lanceolatus* and *E. fuscoguttatus*). On the same hand, Divya et al. (2015) found that *B. cereus*, in combination with *B. subtilis*, *B. licheniformis* and *B. coagulans* are suitable for bioremediation of organic detritus although they did not seem to be naturally present in sufficient population densities in either the water column or sediment. Extracellular products of *B. cereus* together with *B. subtilis* strongly inhibited the growth of pathogenic bacteria *Aeromonas hydrophila* and *Vibrio alginolyticus* isolated from diseased fish (Murillo and Villamil 2011). Isolate F0033 showed highest similarity with *Bacillus jeotgali* (NR_025060.1), *B. boroniphilus* (NR_041275.1), *B. thioparans* (NR_043762.1), *B. subterraneus* (NR_104749.1) and *B. selenatarsenatis* (NR_041465.1) (Table 1). *B. jeotgali* is a probiotic bacterium, firstly isolated from a Korean traditional fermented seafood (Jeotgal) made from salted marine organisms such as fish, molluscs and crustaceans (Yao et al. 2019). There were no prior reports so far on the presence of this probiotic bacterium in rotifer culture but the bacterium was reported to have beneficial effect on survival and development of *Litopenaeus vannamei* larvae (Xue et al. 2016).

The isolate F0032 showed highest similarity to *Vibrio rotiferianus* (NR_118091.1; NR_042081.1; NR_119050.1), *V. campbellii* (NR_113782.1; NR_118258.1) and *V. alginolyticus* (NR_118258.1). Ke et al. (2017) grouped the three bacterial species in the *harveyi* clade of the genus *Vibrio* together with *V. harveyi*, *V. parahaemolyticus*, *V. mytili*, *V. natriegens* and *V. azureus*. According to Chowdhury et al. (2011), the *V. rotiferianus* had $\leq 70\%$ DNA hybridization similarity to its most closely related species, *V. campbellii* and *V. harveyi*, although, they shared 99% identity in 16S rRNA gene sequences. The *V. rotiferianus* is a gram-negative bacterium, was firstly assigned as a new species in 2003 and was named after its isolation source, i.e. from rotifer cultures (Ke et al. 2017). Although *V. rotiferianus* was found in rotifer culture, however, there were no reports on its pathogenicity to fish larvae through transmission from rotifer. The *Vibrio* genus is often reported as inhabitants at low density of marine fish larvae culture even though there were no symptoms of vibriosis appearing (Hannan et al. 2019; Istiqomah et al. 2020). On the other hand, Sahandi et al. (2019) reported the significant use of yeast or probiotics bacteria in reducing growth of *Vibrio*.

Isolate F0035 showed highest similarity to *Staphylococcus warneri* (NR_025922.1), *S. pasteurii* (NR_024669.1; NR_114435.1), *S. epidermidis* (NR_036904.1; NR_113957.1). These three species are member of the bacterial genus *Staphylococcus*, the gram-positive bacteria commonly found as part of the skin flora on humans and animals. *S. warneri* was reported as a resident in skin of rainbow trout and was not pathogenic or has a very low pathogenicity to the marine fish even when injected at very high concentrations (Musharrafieh et al.

2014). So far, there is no report on the presence of *S. warneri* in the culture of rotifer and its transmission from rotifer to fish larvae.

Isolate F0036 and F0037 showed lower similarity to several species of *Alteromonas* retrieved from GenBank. However, using EzBioCloud, F0036 and F0037 were identified as *Alteromonas fortis* with similarity of 99.56% and 99.40%, respectively. Threshold of 98.65% similarity for bacterial delineation using 16S rRNA gene has been proposed previously by Kwon et al. (2014) to differentiate two bacterial species. *Alteromonas* is a genus of *Proteobacteria* found in sea water, either in the open ocean or in the coast. Its cells are curved rods with a single polar flagellum (Barbeyron et al. 2019). Genus *Alteromonas* have been well-documented previously as probiotic in aquaculture (Kesarcodei et al. 2010). This genus was reported to increase the survival of Pacific oyster, *Crassostrea gigas*, when administered in water (Irianto and Aust 2002), inhibit pathogenic bacteria *Vibrio harveyi* and promote the growth and survival of shrimp larvae (Haryanti et al. 2017). *A. fortis* is a non-flagellated bacterium specialized in the degradation of iota-carrageenan (Barbeyron et al. 2019). There were no reports so far on the presence of *A. fortis* in culture of rotifer and fish larvae.

Altogether, we show here that culture medium of rotifer fed FWD containing 5 different bacterial species i.e. *B. cereus* sp. complex (F0031), *V. rotiferianus* (F0032), *B. jeotgali* (F0033), *S. warneri* (F0035), and *A. fortis* (F0036; F0037) in the genus of *Bacillus*, *Vibrio*, *Staphylococcus* and *Alteromonas*. The bacterial species may involve in decomposing the FWD and further become food source for rotifer.

ACKNOWLEDGEMENTS

This work is funded by the Indonesian Ministry of Research and Technology (Kemenristek) /National Agency for Research and Innovation (BRIN) Indonesia to SW.

REFERENCES

- Acién FG, Molina E, Fernández-Sevilla JM, Barbosa M, Gouveia L, Sepulveda C, Bazaes J, Arbib Z. 2017. Economics of microalgae production. In Microalgae-based biofuels and bioproducts. Woodhead Publishing, United Kingdom.
- Akihary CV, Kolondam BJ. 2020. Pemanfaatan gen 16s rRNA sebagai perangkat identifikasi bakteri untuk penelitian-penelitian di Indonesia. *Pharmaco* 9 (1): 16-22.
- Barbeyron T, Zonta E, Le Panse S, Duchaud E, Michel G. 2019. *Alteromonas fortis* sp. nov., a non-flagellated bacterium specialized in the degradation of iota-carrageenan, and emended description of the genus *Alteromonas*. *Int J Syst Evol Microbiol* 69 (8): 2514-2521.
- Bottono EJ. 2010. *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev* 23 (2): 382-398.
- Chandran MN, Iyapparaj P, Moovendhan S, Ramasubburayan R, Prakash Immanuel G. 2014. Influence of probiotic bacterium *Bacillus cereus* isolated from the gut of wild shrimp *Penaeus monodon* in turn as a potent growth promoter and immune enhancer in *P. monodon*. *Fish Shellfish Immunol* 36: 38-45.

- Chowdhury PR, Boucher Y, Hassan KA, Paulsen IT, Stokes HW, Labbate M. 2011. Genome sequence of *Vibrio rotiferianus* strain DAT722. *J Bacteriol* 193 (13): 3381-3382.
- Clément P. 1987. Movements in rotifers: Correlations of ultrastructure and behavior. *Hydrobiol* 147: 339-359.
- Divya M, Aanand S, Srinivasan A, Ahilan. 2015. Bioremediation-An eco-friendly tool for effluent treatment: A review. *Int J Appl Res* 1: 530-537.
- Fialkowska E, Klimek B, Marchlewicz A, Kocerba-Soroka W, Starzycka J, Walczyńska A, Pajdak-Stós A. 2019. Diversity and function of the microbial community under strong selective pressure of rotifers. *J Basic Microbiol* 59 (8): 775-783.
- Fuks G, Elgart M, Amir A, Zeisel A, Turnbaugh PJ, Soen Y, Shental N. 2018. Combining 16S rRNA gene variable regions enables high-resolution microbial community profiling. *Microbiome* 6 (1): 1-13.
- Haché R, Plante S. 2011. The relationship between enrichment, fatty acid profiles and bacterial load in cultured rotifers (*Brachionus plicatilis* L-strain) and Artemia (*Artemia salina* strain Franciscana). *Aquac* 311 (1-4): 201-208.
- Hagiwara A, Kim HJ, Marcial H. 2017. Mass culture and preservation of *Brachionus plicatilis* sp. complex. In: Hagiwara A, Yoshonaga T (eds). *Rotifers Aquaculture, Ecology, Gerontology, and Ecotoxicology*. Springer, Singapore.
- Hagiwara A, Wullur S, Marcial HS, Hirai N, Sakakura Y. 2014. Euryhaline rotifer *Proales similis* as initial live food for rearing fish with small mouth. *Aquaculture* 432: 470-474.
- Hannan MA, Rahman MM, Mondal MN, Deb SC, Chowdhury G, Islam MT. 2019. Molecular Identification of *Vibrio alginolyticus* Causing Vibriosis in Shrimp and Its Herbal Remedy. *Polish J Microbiol* 68 (4): 429-438.
- Haryanti H, Sugama K, Tsumura S, Nishijima R. 2017. Enhance production of black tiger shrimp *Penaeus monodon* postlarvae by probiotic bacterium *Aeromonas* sp. *Indones Fish Res J* 7 (1): 1-6.
- He RP, Feng J, Tian JL, Dong SL, Wen B. 2017. Effects of dietary supplementation of probiotics on the growth, activities of digestive and non-specific immune enzymes 8 in hybrid grouper (*Epinephelus lanceolatus* and *Epinephelus fuscoguttatus*). *Aquac Res* 48: 5782-5790.
- Irianto A, Austin B. 2002. Probiotics in aquaculture. *J Fish Dis* 25 (11): 633-642.
- Istiqomah I, Isnansetyo A. 2020. Review vibriosis management in Indonesian marine fish farming. *E3S Web of Conf*. DOI: 10.1051/e3sconf/202014701001.
- Ke HM, Prachumwat A, Yu CP, Yang YT, Promsri S, Liu KF, Lo CF, Lu MYJ, Lai MC, Tsai IJ, Li WH. 2017. Comparative genomics of *Vibrio campbellii* strains and core species of the *Vibrio* Harveyi clade. *Sci Rep* 7 (1): 1-11.
- Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L. 2010. *Aeromonas macleodii* 0444 and *Neptunomonas* sp. 0536, two novel probiotics for hatchery-reared Greenshell™ mussel larvae, *Perna canaliculus*. *Aquaculture* 309 (1-4): 49-55.
- Kim M, Oh HS, Park SC, Chun J. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 64: 346-351.
- Kotani T. 2017. The current status of the morphological classification of rotifer strain used in aquaculture. In: Hagiwara A, Yoshonaga T (eds). *Rotifers Aquaculture, Ecology, Gerontology, and Ecotoxicology*. Springer, Singapore.
- Lee MC, Park JC, Yoon DS, Choi H, Shin KH, Kim HJ, Hagiwara A, Lee JS. 2019. Lipid metabolism modulation by five different food types in the monogonont marine rotifer *Brachionus koreanus*. *Aquaculture* 503: 596-601.
- Liu Y, Lai Q, Du J, Shao Z. 2017. Genetic diversity and population structure of the *Bacillus cereus* group bacteria from diverse marine environments. *Sci Rep* 7 (1): 1-11.
- Loka J, Sonali SM, Saha P, Devaraj K, Philipose KK. 2016. Use of commercial probiotics for the improvement of water quality and rotifer density in outdoor mass culture tanks. *Indian J Fish* 63 (4): 145-149.
- Loo PL, Chong VC, Vikineswary S, Ibrahim S. 2016. Waste-grown phototrophic bacterium supports culture of the rotifer, *Brachionus rotundiformis*. *Aquac Res* 47 (10): 3029-3041.
- Md SA, Nour AM, Srour TM, Assem SS, Ibrahim HA, El-Sayed HS. 2015. Greenwater, Marine *Bacillus subtilis* HS1 probiotic and symbiotic enriched artemia and rotifers improved European seabass *Dicentrarchus labrax* larvae early weaning length growth, survival, water and bacteriology quality. *Am J Life Sci* 3 (6-1): 45-52.
- Miller RA, Beno SM, Kent DJ, Carroll LM, Martin NH, Boor KJ, Kovac J. 2016. *Bacillus wiedmannii* sp. nov., a psychrotolerant and cytotoxic *Bacillus cereus* group species isolated from dairy foods and dairy environments. *Int J Syst Evol Microbiol* 66 (11): 4744-4753.
- Murillo I, Villamil L. 2011. *Bacillus cereus* and *Bacillus subtilis* used as probiotics in rotifer (*Brachionus plicatilis*) cultures. *J Aquac Res Dev*. DOI: 10.4172/2155-9546.S1-007.
- Musharrafiyah R, Tacchi L, Trujeque J, LaPatra S, Salinas I. 2014. *Staphylococcus warneri*, a resident skin commensal of rainbow trout (*Oncorhynchus mykiss*) with pathobiont characteristics. *Vet Microbiol* 169 (1-2): 80-88.
- Napitupulu HG, Rumengan IF, Wullur S, Ginting EL, Rimper JR, Toloh BH. 2019. *Bacillus* sp. as a decomposition agent in the maintenance of *Brachionus rotundiformis* which uses raw fish as a source of nutrition. *Jurnal Ilmiah Platax* 7 (1): 158-169. [Indonesian].
- Nevejan N, De Schryver P, Wille M, Dierckens K, Baruah K, Van Stappen G. 2018. Bacteria as food in aquaculture: Do they make a difference? *Rev Aquac* 10 (1): 180-212.
- Ogello EO, Wullur S, Hagiwara A. 2019. Blending fishwastes and chicken manure extract as low-cost and stable diet for mass culture of freshwater zooplankton, optimized for aquaculture. *IOP Conf Ser Mater Sci Eng* 567: 012022. DOI: 10.1088/1757-899X/567/1/012022
- Ogello EO, Wullur S, Sakakura Y, Hagiwara A. 2018. Composting fishwastes as low-cost and stable diet for culturing *Brachionus rotundiformis* Tschugunoff (Rotifera): Influence on water quality and microbiota. *Aquaculture* 486: 232-239.
- Ogello EO, Wullur S, Yoshitaka S, Hagiwara A. 2020. Dietary value of waste-fed rotifer *Brachionus rotundiformis* on the larval rearing of Japanese whiting *Sillago japonica*. *E3S Web of Conferences*. DOI: 10.1051/e3sconf/202014701005.
- Onianwah IF, Stanley HO, Oyakhire MO. 2018. Microorganisms in sustainable aquaculture development. *Global Adv Res J Microbiol* 7 (8): 127-131.
- Rumengan IFM, Sulung M, Lantiunga Z, Kekenusa J. 2016. Morfometri Rotifer *Brachionus rotundiformis* strain SS asal tambak Minanga dan tambak Watuliny Sulawesi Utara yang dikultur pada salinitas yang berbeda. *Jurnal Riset Akuakultur* 2 (2): 221-229. [Indonesian]
- Sahandi J, Sorgeloos P, Xiao H, Wang X, Qi Z, Zheng Y, Tang X. 2019. The use of selected bacteria and yeasts to control *Vibrio* spp. in live food. *Antibiotics* 8 (3): 1-16.
- Sousa AM, Pereira MO, Lourenço A. 2015. MorphoCol: An ontology-based knowledgebase for the characterisation of clinically significant bacterial colony morphologies. *J Biomed Inform* 55: 55-63.
- Sultani M, Ghosh K, Hoseinifar SH, Kumar V, Lymbery AJ, Roy S, Ringo E. 2019. Genus *Bacillus*, promising probiotics in aquaculture: Aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. *Rev Fish Sci Aquac* 27 (3): 331-379.
- Vargas-Albore F, Porchas-Cornejo MA, Martinez-Porshas M, Villalpando E, Gollas-Galvan T, Martinez-Cordova LR. 2017. Bacterial biota of shrimp intestine is significantly modified by the use of a probiotic mixture: A high throughput sequencing approach. *Helgoland Mar Res* 71 (5): 1-10.
- Wantania LL, Wullur S, Ginting EL, Mantiri DM, Undap SL, Sumilat DA, Gerung GS. 2019. Isolation and amplification of 16S rRNA gen of associated microbial isolates in red algae *Kappaphycus alvarezii* from Belang, Southeast Minahasa Regency, North Sulawesi. *Jurnal Ilmiah Platax* 7 (1): 220-226. [Indonesian]
- Wati M, Imanto PT. 2016. Kultur rotifer dengan beberapa jenis pakan dan kombinasinya. *Jurnal Riset Akuakultur* 4 (3): 349-356. [Indonesian]
- Wondal B, Ginting EL, Warouw V, Wullur S, Tilaar SO, Tilaar FF. 2019. Isolasi bakteri laut dari perairan Malalayang, Sulawesi Utara. *Jurnal Pesisir dan Laut Tropis* 7 (3): 183-189. [Indonesian]
- Wullur S, Ginting EL, Waraow V, Rumengan IFM, Ogello EO, Hagiwara A. 2019. Growth response of rotifers on a bacterial-based diet made from fishwastes. *IOP Conf Ser Mater Sci Eng* 567: 012030. DOI: 10.1088/1757-899X/567/1/012030.
- Wullur S, Kumagai S, Sakakura Y, Hagiwara A. 2018. Assessment of different minute zooplankton in the larval rearing of rusty angelfish *Centropyge ferrugata*. *AACL Bioflux* 11 (5): 1495-1501.
- Wullur S, Sakakura Y, Hagiwara A. 2011. Application of the minute monogonont rotifer *Proales similis* de Beauchamp in larval rearing of seven-band grouper *Epinephelus septemfasciatus*. *Aquaculture* 315 (3-4): 355-360.

- Wullur S, Yoshimatsu T, Tanaka H, Ohtani M, Sakakura Y, Kim HJ, Hagiwara A. 2013. Ingestion by Japanese eel *Anguilla japonica* larvae on various minute zooplanktons. *Aquac Sci* 61 (4): 341-347.
- Wullur S. 2017. Rotifer dalam Perspektif Marikultur. LPPM Press, Manado. [Indonesian]
- Xue M, Wen C, Liang H, Ding M, Wu Y, Li X. 2016. In vivo evaluation of the effects of commercial *Bacillus probiotics* on survival and development of *Litopenaeus vannamei* larvae during the early hatchery period. *Aquac Res* 47 (5): 1661-1669.
- Yao Z, Meng Y, Le HG, Kim JA, Kim JH. 2019. Isolation of *Bacillus subtilis* SJ4 from Saeu (Shrimp) jeotgal, a Korean fermented seafood, and its fibrinolytic activity. *J Korean Soc Microbiol Biotechnol* 47 (4): 522-529.
- Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67 (5): 1613.

Molecular identification of bacteria isolated from culture medium of rotifer fed on fishery waste diet

ORIGINALITY REPORT

18%

SIMILARITY INDEX

9%

INTERNET SOURCES

16%

PUBLICATIONS

6%

STUDENT PAPERS

PRIMARY SOURCES

1

vkm.no

Internet Source

1%

2

Einar Ringø. "Probiotics in shellfish aquaculture", *Aquaculture and Fisheries*, 2020

Publication

1%

3

studentsrepo.um.edu.my

Internet Source

1%

4

P. R. Chowdhury. "The first genome sequence of *Vibrio rotiferianus*: strain DAT722", *Journal of Bacteriology*, 05/06/2011

Publication

1%

5

othes.univie.ac.at

Internet Source

1%

6

Marièma Sarr, Cheikh Ibrahima Lo, Mamadou Lamine Tall, Amael Fadlane et al. "Taxonogenomics description of *Bacillus dakarensis* sp. nov., *Bacillus sinesaloumensis* sp. nov., and *Bacillus massiliogabonensis* sp. nov., three new species isolated from human stools", *New*

1%

-
- | | | |
|----|---|-----|
| 7 | www.slideshare.net
Internet Source | 1% |
| 8 | Qionglin Huang, Zhonggang Duan, Jinfen Yang, Xinye Ma, Ruoting Zhan, Hui Xu, Weiwen Chen. "SNP Typing for Germplasm Identification of <i>Amomum villosum</i> Lour. Based on DNA Barcoding Markers", PLoS ONE, 2014
Publication | 1% |
| 9 | Submitted to Georgia State University
Student Paper | 1% |
| 10 | M.-J. Jung. "Lentibacillus jeotgali sp. nov., a halophilic bacterium isolated from traditional Korean fermented seafood", INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 08/07/2009
Publication | <1% |
| 11 | Submitted to University of Florida
Student Paper | <1% |
| 12 | Nehaya Al-Karablieh. "Antimicrobial Activity of <i>Bacillus Persicus</i> 24-DSM Isolated from Dead Sea Mud", The Open Microbiology Journal, 2017
Publication | <1% |
-

13

Einar Ringø, Hien Van Doan, Soon Ho Lee, Mehdi Soltani, Seyed Hossein Hoseinifar, Ramasamy Harikrishnan, Seong Kyu Song. "Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture", *Journal of Applied Microbiology*, 2020

Publication

<1%

14

Rico Rico Taareluan, Letha L Wantania, Elvy L Ginting, Remy E.P. Mangindaan et al. "AMPLIFIKASI GEN 16S-rRNA BAKTERI EPIFIT PADA ALGA MERAH *Kappaphycus alvarezii*", *JURNAL PESISIR DAN LAUT TROPIS*, 2020

Publication

<1%

15

T. H. Noble, H. H. Truong, A. Rombenso, C. J. Simon, N. M. Wade, J. W. Wynne. "Effect of sample type and the use of high or low fishmeal diets on bacterial communities in the gastrointestinal tract of *Penaeus monodon*", *Applied Microbiology and Biotechnology*, 2021

Publication

<1%

16

Pepen Supendi, Sri Widiyantoro, Abdul Muhari, Nicholas Rawlinson et al. "Potential megathrust earthquakes and tsunamis off the southern coast of West Java, Indonesia", *Research Square*, 2020

Publication

<1%

17

Greeshma James, Bini C. Das, Seena Jose, Rejish Kumar V.J. "Bacillus as an aquaculture friendly microbe", Aquaculture International, 2021

Publication

<1%

18

"Rotifers", Springer Science and Business Media LLC, 2017

Publication

<1%

19

www.longdom.org

Internet Source

<1%

20

portlandpress.com

Internet Source

<1%

21

Submitted to University of Science and Technology

Student Paper

<1%

22

real.mtak.hu

Internet Source

<1%

23

Huei-Mien Ke, Dang Liu, Yoshitoshi Ogura, Tetsuya Hayashi, Henryk Urbanczyk, Isheng J. Tsai. " Tracing Genomic Divergence of Bacteria in the Harveyi Clade ", Journal of Bacteriology, 2018

Publication

<1%

24

Sukanta K. Nayak. " Multifaceted applications of probiotic species in aquaculture with special reference to ", Reviews in Aquaculture, 2020

<1%

25

"Rotifer Symposium V", Springer Science and Business Media LLC, 1989

Publication

<1%

26

Edyta Fiałkowska, Beata Klimek, Ariel Marchlewicz, Wioleta Kocerba-Soroka et al. "Diversity and function of the microbial community under strong selective pressure of rotifers", Journal of Basic Microbiology, 2019

Publication

<1%

27

www.bacterio.net

Internet Source

<1%

28

"Antarctic Seaweeds", Springer Science and Business Media LLC, 2020

Publication

<1%

29

www.pnas.org

Internet Source

<1%

30

Miaoyu Li, Bingwen Xi, Ting Qin, Kai Chen, Jun Xie. "Isolation and characterization of AHL-degrading bacteria from fish and pond sediment", Journal of Oceanology and Limnology, 2018

Publication

<1%

31

www.saniclad.co.uk

Internet Source

<1%

"Contaminants in Drinking and Wastewater

32

Sources", Springer Science and Business Media
LLC, 2021

Publication

<1%

33

Haryanti Haryanti, Ketut Sugama, Seiichi
Tsumura, Roshitaka Nishijima. "ENHANCE
PRODUCTION OF BLACK TIGER SHRIMP
Penaeus monodon POSTLARVAE BY
PROBIOTIC BACTERIUM Alteromonas sp.",
Indonesian Fisheries Research Journal, 2017

Publication

<1%

34

kundoc.com

Internet Source

<1%

35

revistas.reduc.edu.cu

Internet Source

<1%

36

Yan Cai, Wei Yuan, Shifeng Wang, Weiliang
Guo, An Li, Yue Wu, Xin Chen, Zhuling Ren,
Yongcan Zhou. "In vitro screening of putative
probiotics and their dual beneficial effects: To
white shrimp (*Litopenaeus vannamei*) postlarvae
and to the rearing water", Aquaculture, 2018

Publication

<1%

37

Sarah Finke, Annette Fagerlund, Veronika
Smith, Veronica Krogstad et al. "Bacillus
thuringiensis CbpA is a collagen binding cell
surface protein under c-di-GMP control", The
Cell Surface, 2019

Publication

<1%

38

Alexandre Andrade. "Molecular characterization of a putative heat shock protein cognate gene in *Rhynchosciara americana*", Chromosome Research, 09/19/2009

Publication

<1%

39

journal.frontiersin.org

Internet Source

<1%

40

www.hatcheryfeed.com

Internet Source

<1%

41

Atsushi Hagiwara, Helen S. Marcial. "The use of non-*Brachionus plicatilis* species complex rotifer in larviculture", Hydrobiologia, 2018

Publication

<1%

42

Wen-Ming Chen, Ting-Hsuan Chang, Der-Shyan Sheu, Li-Cheng Jheng, Shih-Yi Sheu. "Rhodobacter ruber sp. nov., isolated from a freshwater pond", Research Square, 2021

Publication

<1%

43

Rotifer Symposium VI, 1993.

Publication

<1%

Exclude quotes

On

Exclude matches

Off

Exclude bibliography

On