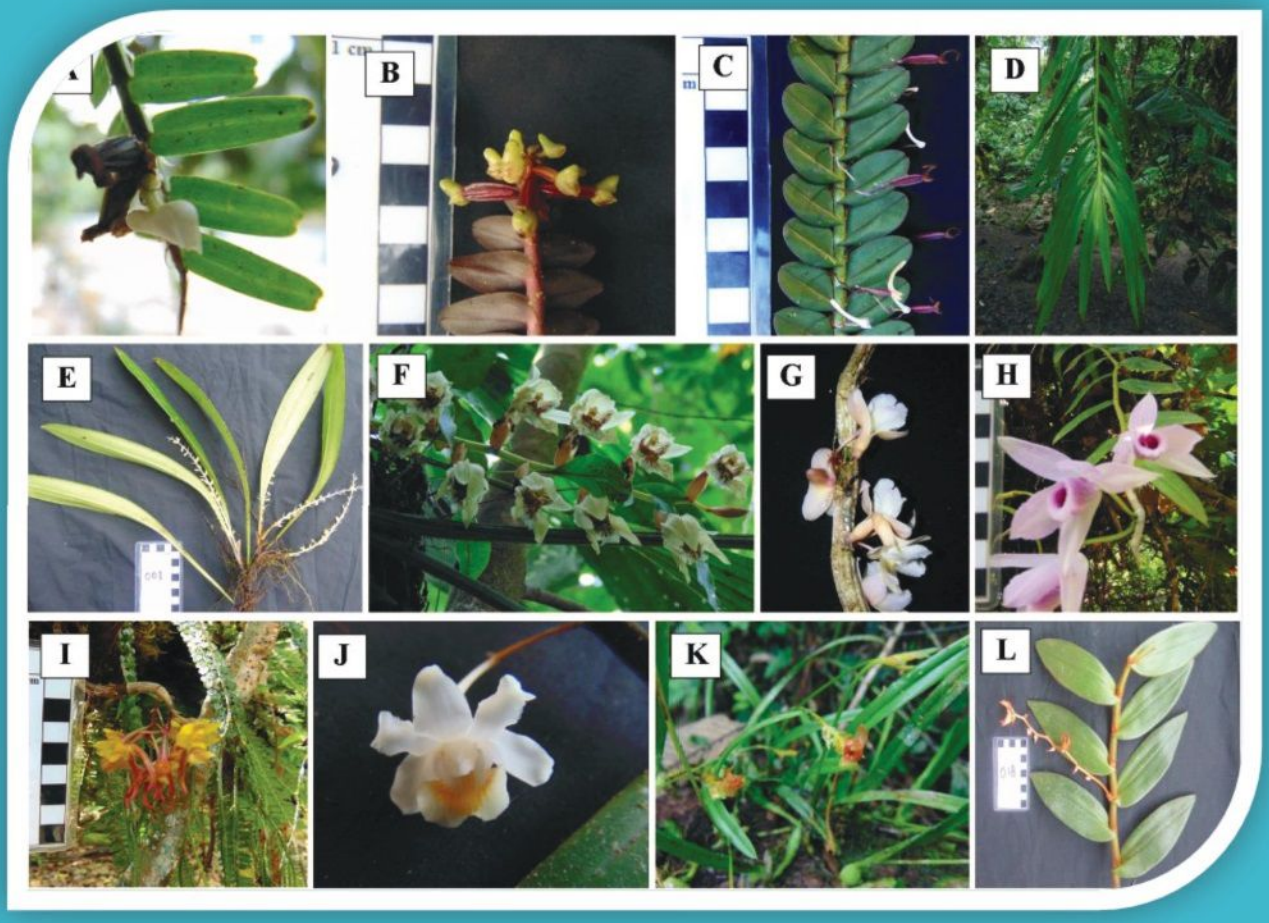
LIPI

P-ISSN 0126-1754
E-ISSN 2337-8751
Terakreditasi Peringkat 2
21/E/KPT/2018
Volume 18 Nomor 3, Desember 2019

Berita Biologi

Jurnal Ilmu-ilmu Hayati



Berita Biologi	Vol. 18	No. 3	Hlm. 255 - 375	Bogor, Desember 2019	ISSN 0126-1754

Pusat Penelitian Biologi - LIPI

BERITA BIOLOGI

Vol. 18 No. 3 Desember 2019
Terakreditasi Berdasarkan Keputusan Direktur Jendral Penguatan Riset dan Pengembangan, Kemenristekdikti RI
No. 21/E/KPT/2018

Alamat (*Address*)
Pusat Penelitian Biologi-LIPI
Kompleks Cibinong Science Center (CSC-LIPI)
Jalan Raya Jakarta-Bogor KM 46,
Cibinong 16911, Bogor-Indonesia
Telepon (021) 8765066 - 8765067
Faksimili (021) 8765059
Email: berita.biologi@mail.lipi.go.id
jurnalberitabiologi@yahoo.co.id
jurnalberitabiologi@gmail.com

Keterangan foto cover depan: Jenis anggrek epifit di kaki gunung Liangpran.
(*Notes of cover picture*): (*The epiphytic orchids in the foothill of Mount Liangpran)* sesuai dengan halaman 312 (*as in page 312*).

P-ISSN 0126-1754
E-ISSN 2337-8751
Terakreditasi Peringkat 2
21/E/KPT/2018
Volume 18 Nomor 3, Desember 2019

LIPI

Berita Biologi

Jurnal Ilmu-ilmu Hayati

Berita Biologi	Vol. 18	No. 3	Hlm. 255 – 375	Bogor, Desember 2019	ISSN 0126-1754

Pusat Penelitian Biologi - LIPI

Ucapan terima kasih kepada
Mitra Bebestari nomor ini
18(3) – Desember 2019

Prof. Dr. Mulyadi
(Taksonomi Copepoda, Pusat Penelitian Biologi-LIPI)

Prof. Dr. Tukirin Partomihardjo
(Ekologi Hutan dan Biogeografi Pulau, Ketua Forum Pohon Langka Indonesia)

Prof. Dr. Ir. Sulistiono, M.Sc.
(Biologi Perikanan, FPIK - Institut Pertanian Bogor)

Dr. Mirza Kusrini
(Herpetologi, Ekologi Satwaliar, Fakultas Kehutanan - Institut Pertanian Bogor

Dr. Ir. Praptiwi, M.Agr.
(Fitokimia, Pusat Penelitian Biologi-LIPI)

Dr. Iwan Saskiawan
(Mikrobiologi, Pusat Penelitian Biologi-LIPI)

Deden Girmansyah, S.Si., M.Si.
(Taksonomi Tumbuhan, Pusat Penelitian Biologi-LIPI)

Reni Ambarwati, S.Si., M.Sc.
(Taksonomi Hewan, FMIPA- Universitas Negeri Surabaya

Ucu Yanu Arbi M.Si.
(Zoologi, Loka Konservasi Biota Laut Bitung – LIPI

Dr. Ir. Wartika Rosa Farida
(Nutrisi dan Penangkaran satwaliar, Pusat Penelitian Biologi-LIPI)

Dr. Lina S Juswara, S.P., M.Sc.
(Taksonomi Tumbuhan, Pusat Penelitian Biologi-LIPI)

Dr. rer. nat. Ayu Savitri Nurinsiyah
(Taksonomi Moluska, Pusat Penelitian Biologi-LIPI)

Toga Pangihotan Napitupulu, M.Sc.
(Mikrobiologi Lingkungan, Pusat Penelitian Biologi-LIPI)

Dr. Nuning Argo Subekti, SP, M.Sc.
(Pemuliaan dan Genetika Tanaman, Pusat Penelitian Dan Pengembangan Tanaman Pangan)

Berita Biologi
LIPI
Jurnal Ilmu-ilmu Hayati

DOI: 10.14203/beritabiologi.v18i3.3578
P-ISSN 0126-1754
E-ISSN 2337-8751

IDENTIFICATION AND PATHOGENICITY TEST OF SOME BACTERIA ISOLATED FROM WILD AND FARMED SPINY LOBSTER *Panulirus homarus*

[Identifikasi dan Uji Patogenisitas Bakteri yang Diisolasi dari Lobster *Panulirus homarus* Alam dan Budidaya]

Sudewi*✉, Zeny Widiastuti, Indah Mastuti and Ketut Mahardika

Institute for Mariculture Research and Fisheries Extension (IMRAFE)
Dusun Gondol, Desa Penyabangan, Kec. Gerokgak, Kab. Buleleng-Bali. 81101
email: dewigrim@gmail.com

ABSTRAK

Populasi bakteri dalam budidaya lobster dapat memberikan efek yang menguntungkan maupun merugikan, tergantung pada kondisi yang ada. Oleh karena itu, dilakukan penelitian ini untuk mengidentifikasi dan melakukan uji patogenisitas beberapa isolat bakteri yang berasal dari spiny lobster *Panulirus homarus* alam dan budidaya. Sampel lobster dewasa dari hasil budidaya diperoleh dari Pangandaran dan Lombok, sementara lobster alam dikoleksi dari Lombok, dengan masing-masing lima ekor lobster. Bakteri diisolasi dari usus bagian tengah, insang, hepatopankreas, dan dari daging lobster. Identifikasi bakteri dilakukan menggunakan metode molekuler. Uji patogenisitas dilakukan melalui injeksi intramuskuler 0,1 ml suspensi bakteri dengan kepadatan 7×10^6cfu/ml untuk setiap delapan isolat bakteri terhadap masing-masing tiga ekor lobster yang sehat. Penelitian kami berhasil mengidentifikasi enam isolat bakteri berdasarkan kemiripan sekuens nukleotida yang tinggi dengan *Shewanella algae, Bacillus firmus, Vibrio alginolyticus, Tenacibaculum lutimaris, Pseudomonas* sp. dan *Vibrio* sp., sedangkan dua isolat yang lain kami nyatakan sebagai *Unidentified-1* dan *Unidentified-2* karena kemiripan sekuens nukleotidanya rendah (< 97%). Uji patogenisitas menunjukkan tidak adanya mortalitas lobster yang diinjeksi dengan bakteri-bakteri tersebut. Hal tersebut kemungkinan disebabkan oleh dosis injeksi yang rendah untuk dapat menyebabkan infeksi bakteri terutama untuk *Vibrio*. Kemungkinan lain yaitu bakteri tersebut memang tidak bersifat patogen terhadap lobster atau bahkan memiliki potensi sebagai bakteri probiotik.

Kata kunci: identifikasi, patogenisitas, bakteri, *Panulirus homarus,* spiny lobster.

ABSTRACT

The bacterial populations in the farming of spiny lobster could have either beneficial or harmful effects depending on the prevailing conditions. We designed this study to identify and to perform a pathogenicity test of some bacteria isolated from wild and farmed spiny lobsters *Panulirus homarus*. The adult farmed lobsters were obtained from Pangandaran and Lombok coastal areas, while the wild lobsters were collected from Lombok, with five lobsters for each location. The bacteria were isolated from the midgut, gill, hepatopancreas, and muscle tissues of the lobsters. The identification of the bacteria was carried out by molecular methods. Pathogenicity test was performed by intramuscular injection of 0.1 ml bacterial suspensions at the density of 7×10^6cfu/ml into each three adult apparently healthy lobsters for every eight bacterial isolates. Our study identified six bacterial isolates that exhibited high homology of a nucleotide sequence with *Shewanella algae, Bacillus firmus, Vibrio alginolyticus, Tenacibaculum lutimaris, Pseudomonas* sp. and *Vibrio* sp., while two isolates were reminded unidentified due to low nucleotide similarities (< 97%). The pathogenicity test showed that there was no mortality of lobsters injected with those bacterial isolates. This may because the dose of injection was too low to induce bacterial infection particularly for *Vibrio*, or the bacteria were not pathogenic for lobster or even have the potency as probiotic bacteria.

Keywords: identification, pathogenicity, bacteria, *Panulirus homarus,* spiny lobster.

INTRODUCTION

Microbial population in aquaculture plays an essential role, such as being a subject of refined balance in the relationship between the host and environment. Disease outbreaks which may affect severely the host could occur when the balance is disturbed. In the aquaculture environment, microbia access the host by colonizes proliferation in the gills and it's spreading, affecting in both harmful and beneficial ways depending on the existing conditions (Immanuel *et al.*, 2006).

In crustaceans, colonization of microbial in the intestine has great consequence in healthy conditions (Immanuel *et al.*, 2006). *Vibrio* is the most frequent genera associated with crustaceans causing considerable economic losses. Various strains of vibrios are known to cause diseases in numerous crustaceans such as prawn and lobster. *Vibrio harveyi* had been detected by PCR method in live lobsters *Panulirus homarus* of the size ranging from 45 mm to 50 mm in India (Leslie *et al.*, 2013).

Data on the microbial of spiny lobster *P. homarus* had been available in live transportation process (Immanuel *et al.*, 2006). In addition, *Vibrio* spp. in the hemolymph of *P. homarus* had been detected by molecular method by Raissy *et al.* (2011). These several previous studies had been done only to know the bacterial communities of

*Kontributor Utama
*Diterima: 21 Mei 2018 - Diperbaiki: 7 Januari 2019 - Disetujui: 30 Oktober 2019

P. homarus, not on the pathogenicity of those bacteria. To date, information on the pathogenicity of some bacteria isolated from wild and farmed *P. homarus* particularly in Indonesia still lacks and poorly known. Identification of bacteria from wild and farmed lobster had been done only in Lombok by using biochemical methods (Lasmika, 2015), despite the fact that molecular characterization methods are currently widely used for analysis of various bacterial species (Maiti *et al*., 2009). Therefore, the present study was carried out to perform bacterial identification by molecular methods to obtain more reliable data, and to conduct pathogenicity test of some bacteria isolated from wild and farmed spiny lobster *P. homarus*.

MATERIALS AND METHODS

Bacterial isolation

Five wild lobsters were randomly captured from Lombok coastal areas by fishermen for this study purpose, while five farmed lobsters were obtained by random sampling from net cages each in Lombok and Pangandaran, in 2016. The average weight of the wild lobsters from Lombok was 84.34±20.69 g, while the average weight of the farmed lobsters from Lombok was 211.6±34.16 g and from Pangandaran was 128.95±30.07 g. All the samples were transported by dry method to Institute for Mariculture Research and Fisheries Extension (IMRAFE), Gondol-Bali for bacterial isolation and identification.

The gill, mid gut, hepatopancreas, and muscle tissues of the lobsters were aseptically collected, liquefied, and homogenized. The tissue homogenates were quantitatively diluted (serial 10-fold dilutions) with sterile seawater, and 100 µl of each sample was then separately spread plated onto Marine Agar (MA) and TCBS Agar (Payne *et al*., 2007). Subsequently, they were incubated at 25 °C for 24 h. Then, the bacterial colonies were separated according to its characteristics including their size, shape, structure, edge, surface, and color. Each colony type was streaked onto MA or TCBS agar, and purified by a second or a third plating using the same medium. Each purified colony was then inoculated into marine broth, incubated at 25 °C for 24 h, and harvested by centrifugation at 6000 rpm for 5 minutes to obtain bacterial pellet. The pellets were kept at -20 °C until used for genomic DNA extraction.

Random Amplified Polymorphic DNA (RAPD)-PCR Assay

Molecular identification of bacterial isolates was performed by PCR-based DNA finger printing method that was random amplified polymorphic DNA (RAPD) using random primers to detect variations in the sequence of DNA at sites in the genome (Maiti *et al*., 2009). Bacterial pellets of 8 apparently different colonies of bacteria were selected for RAPD analysis. Isolation of bacterial genomic DNA was done using Presto Mini gDNA Bacteria kit (Geneaid) according to the manufacturer's manual. The extracted DNA was then amplified for RAPD-PCR assay using universal primer 2AAM2 (Haryanti *et al*., 2011) to determine its genetic variance. The oligonucleotide sequences of the primer were 5'- CTG CGA CCC AGA GCG -3'. PCR reaction for the RAPD analysis was contained 5x Green GoTaq reaction buffer (PROMEGA), dNTP (10mM), primer 2AAM2 (25µM), MgCl (25mM), GoTaq DNA Polymerase (5u/µl), NFW, and template DNA with a total reaction of 10 µl. Thermal cycling conditions were as follows: denaturation at 95 ^{o}C for 2 min, 2 cycles of the first round at 95 ^{o}C for 15 s, 45 ^{o}C for 15 s and 70 ^{o}C for 1 min. The second round (38 cycles) at 94 ^{o}C for 5 s, 45 ^{o}C for 15 s and 70 ^{o}C for 30 s. Final extension at 70 ^{o}C for 3 min. The PCR products were then subjected to gel electrophoresis (2% of agarose) in 1x TBE buffer, at 100 V and 200 mA for 90 min. DNA was stained using 0.5 µg/ml ethidium bromide, measured using 100 bp DNA ladder (Solis Biodyne) and visualized under a UV transiluminator. Bacterial isolates showed different pattern of DNA band suggested its genetic variance that indicated different species of bacteria.

PCR for gene sequencing

Each genomic DNA of eight different bacterial isolates (based on RAPD analysis) was subjected to PCR using universal primer set for gene sequencing. A fragment of the 16S rRNA gene was PCR amplified for each genomic DNA sample using

forward primer 27F (5' - AGA GTT TGA TCM TGG CTC AG - 3') and reverse primer 1492R (5' - ACG GYT ACC TTG TTA CGA CTT - 3'), as described by Hongoh *et al*. (2003). The genomic DNA was amplified in 10 x PCR buffer, dNTP (10mM), Taq DNA Polymerase (QIAGEN) (2.5 u/reaction), primer 27F (10 µM), primer 1492R (10 µM), NFW, and template DNA to a total volume of 50 µl. PCR conditions involved initial denaturation at 94 °C for 3 min, denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. Visualization of the PCR products on a 1.5 % agarose gel was made to check succesfull amplification of the 16S rRNA fragment. The remaining PCR products were prepared for gene sequencing by purification using a QIAquick PCR purification kit (Qiagen). Gene sequencing was accomplished for the forward and reverse directions using the 27F and 1492R primers, respectively, by 1st BASE DNA sequencing Malaysia. Approximately 1492 bp of unambiguous sequence was attained in both directions, and species identification was finished by comparing the sequences obtained in this study to the GenBank database by the BLASTn program (blast.ncbi.nlm.nih.gov/Blast.cgi). Minimal matches were at ≥ 97% identity (Rungrassamee *et al*., 2014).

Pathogenicity test

A random selection of 24 apparently healthy *P. homarus* were distributed in nine plastic aquaria (75×50×50 cm) containing 80 L of sea water, stocked at a density of three lobsters per aquarium. The tested lobsters were acclimatized in the aquaria for two days. The aquaria were supplied with sea water and continuous aeration was provided by using a blower. They fed on trash fish at satiation, at 08:00 am and 15:00 pm. The pathogenicity test was conducted for eight bacterial isolates that were Pa-U5-2, Pa-I3-1, Pa-U4-1 and Pa-Hp5-1 which were isolated from wild lobsters from Pangandaran, and L-B-U1, L-A-I2-4, L-B-I1-2, and L-A-D1 which were isolated from wild and farmed lobsters from Lombok. Suspension of the bacteria was prepared by culturing of each bacterial isolates for 24 h in Marine Broth. Each lobster from group 1 to group 8 was inoculated intra-musculary (IM) with 0.1 ml of each prepared bacterial suspension (7×10^6cfu/ml). The ninth group of lobster was inoculated IM with 0.1 ml of sterile Phospat Buffer Saline (PBS) and this group served as negative control. The pathogenicity test was performed for 5 days.

RESULTS

Molecular identification of the bacterial isolates

In this study, RAPD-PCR fingerprints using universal primer 2AAM2 generated eight different RAPD patterns which revealed that the eight bacterial isolates had different genetic variations indicated by different size of DNA band measured using 100 bp DNA ladder (Fig. 1). These results implied that these isolates were different species of bacteria. Therefore, all the isolates were then subjected to PCR using primer set 27F and 1492R (Fig. 2) in order to determine the identity of the bacteria by performing gene sequencing analysis.

Results of nucleotide sequence analysis using BLAST search were presented in Table 1. Gene sequences of Pa-U5-2 isolate revealed 100% nucleotide similarities with the sequences of *Shewanella algae* strain CUMBSA 01-A1 (Gen Bank accession no. KY072884.1). Therefore, Pa-U5-2 that isolated from the mid gut of the farmed lobster from Pangandaran was ultimately identified as *Shewanella algae.* Bacterial solates Pa-Hp5-1 and Pa-I3-1 that were isolated from the hepatopancreas and the gill of the farmed lobsters form Pangandaran were exhibited 100% and 97% identity with *Bacillus firmus* strain TP6 and *Vibrio alginolyticus* strain H4C2, respectively. Furthermore, L-B-U1 and L-B-I1-2 that were isolated from the mid gut and the gill of the farmed lobsters from Lombok were chategorized as unidentified-1 and -2 because homology of its nucleotide sequences was <97%. Lastly, L-A-I2-4 and L-A-D1 that isolated from the gill and muscle tissues of the wild lobsters from Lombok, and Pa-U4-1 that isolated from the mid gut of the farmed lobster from Pangandaran were determined as *Tenacibaculum lutimaris, Pseudomonas* sp. and *Vibrio* sp., respectively, with their similarity of nucleotide sequence 98%, 99% and 100% respectively.

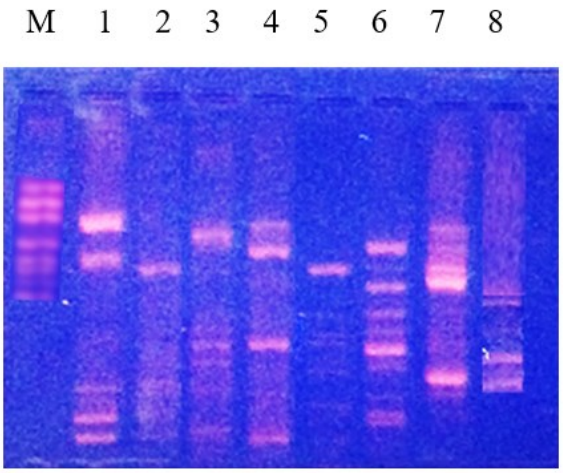


Figure 1. Results of PCR on genomic DNA of eight bacterial isolates from wild and farmed lobster *Panulirus homarus* using primer 2AAM2 for RAPD analysis. M: Marker DNA Ladder 100 bp. 1-8: isolate ID: Pa-U5-2, Pa-Hp5-1, Pa-I3-1, L-B-U1, L-A-I2-4, L-B-I1-2, L-A-D1, and Pa-U4-1. All bacterial isolates showed different genetic diversity that indicated by the differences in the DNA band patterns. It could be stated that these isolates were different species of bacteria (*Hasil PCR dari DNA genom 8 isolat bakteri lobster Panulirus homarus alam dan budidaya menggunakan primer 2AAM2 untuk analisis RAPD. M: Marker DNA Ladder 100 bp. 1-8: identitas isolat: Pa-U5-2, Pa-Hp5-1, Pa-I3-1, L-B-U1, L-A-I2-4, L-B-I1-2, L-A-D1, dan Pa-U4-1. Seluruh isolat bakteri tersebut menunjukkan keragaman genetika yang berbeda yang ditandai dengan pola pita DNA yang berbeda. Dengan demikian dapat dinyatakan bahwa delapan isolat bakteri tersebut merupakan spesies bakteri yang berbeda*)

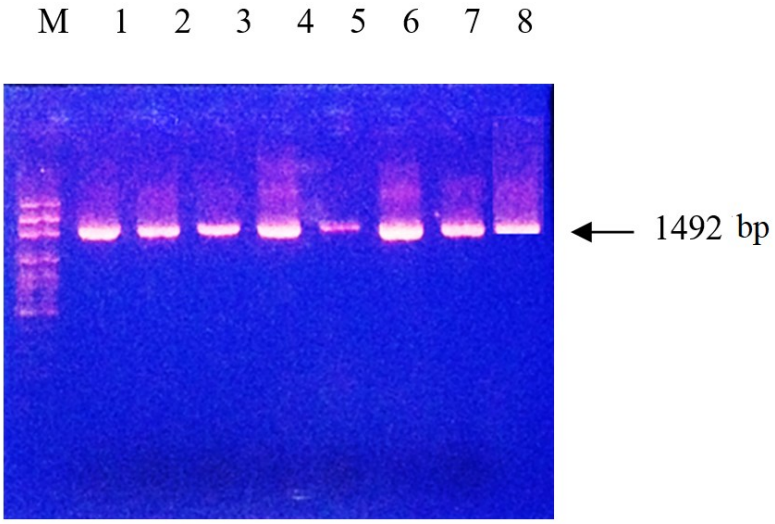


Figure 2. Results of PCR on fragment of the 16S rRNA gene for eight bacterial isolates from wild and farmed lobster *Panulirus homarus* using universal forward primer 27F and reverse primer 1492R for gene sequencing. M : Marker DNA Ladder 100 bp. 1-8: isolate ID: Pa-U5-2, Pa-Hp5-1, Pa-I3-1, L-B-U1, L-A-I2-4, L-B-I1-2, L-A-D1, and Pa-U4-1. Approximately 1492 bp of unambiguous sequence was attained in both directions [*Hasil PCR untuk fragmen gen 16S rRNA dari delapan isolat bakteri lobster Panulirus homarus menggunakan primer universal 27F dan 1492R untuk sekuensing gen. M : Marker DNA Ladder 100 bp. 1-8: identitas isolat: Pa-U5-2, Pa-Hp5-1, Pa-I3-1, L-B-U1, L-A-I2-4, L-B-I1-2, L-A-D1, dan Pa-U4-1. Sekuen DNA yang jelas dengan ukuran sekitar 1492 bp diperoleh dari kedua arah (27F dan 1492R)*]

Table 1. Sequence identity of eight bacterial isolates from wild and farmed lobster *Panulirus homarus* based on analysis of homologous sequences using BLASTn program. The bacteria were chategorized as "unidentified" when the homologous sequences (similarity) was <97% (*Identitas sekuen delapan isolat bakteri dari lobster Panulirus homarus alam dan budidaya berdasarkan analisis kemiripan sekuen menggunakan program BLASTn. Isolat bakteri dinyatakan sebagai "tidak teridentifikasi" jika kemiripan sekuens <97%*)

No.	Isolate ID (*Identitas isolat*)	Closest species (*Spesies terdekat*)	Database accession number (*Nomor akses database*)	Similarity (*kemiripan sekuen*)
1	Pa-U5-2	*Shewanella algae* strain CUMBSA 01-A1 16S ribosomal RNA gene	KY072884.1	693/693 (100%)
2	Pa-Hp5-1	*Bacillus firmus* strain TP6 16S ribosomal RNA gene	KX870874.1	373/373 (100%)
3	Pa-I3-1	*Vibrio alginolyticus* strain H4C2 16S ribosomal RNA gene	KX966482.1	567/587 (97%)
4	L-B-U1*	*Vibrio alginolyticus* partial 16S rRNA gene	FR687009.1	140/146 (96%)
5	L-A-I2-4	*Tenacibaculum lutimaris* strain HNS041 16S ribosomal RNA gene	JN128275.1	184/188 (98%)
6	L-B-I1-2**	*Lysinibacillus fusiformis* strain Ma-Su CE-CRI 2 16S ribosomal RNA gene	GQ501071.1	986/1127 (87%)
7	L-A-D1	*Pseudomonas* sp. TSWCW16 16S ribosomal RNA gene	GQ284461.1	922/935 (99%)
8	Pa-U4-1	*Vibrio* sp. P15 16S ribosomal RNA gene	KR075019.1	760/760 (100%)

Notes (*keterangan*): * Unidentified-1 (*tidak teridentifikasi-1*), ** Unidentified-2 (*tidak teridentifikasi-2*)

Pathogenicity test

The mortality rate was 0% in all groups in which lobsters were injected IM with the bacteria as well as in the control group. Pathogenicity test using eight bacterial isolates for five days also demonstrated no symptoms of bacterial infection in the tested lobsters (Table 2).

DISCUSSION

In our study, we have executed RAPD-PCR molecular methods to differentiate eight bacterial isolates from the wild and farmed lobster. RAPD-PCR was exploited using primer 2AAM2 produced well distinguishable amplicons. Therefore, the isolates were then subjected to PCR for gene sequencing, and followed by analysis of homologous sequence by BLASTn program to know the identity the bacterial isolates.

The present study identified some bacteria inhabited in wild and farmed spiny lobster *P. homarus.* The mid gut was inhabited by *Shewanella alga* and *Vibrio* sp. The hepatopancreas was populated by *Bacillus firmus.* The gill was colonized by *Vibrio alginolyticus and Tenacibaculum lutimaris,* while the muscle was dwelled by *Pseudomonas* sp. Other species of bacteria found by Lasmika (2015), included *Vibrio damsella, V. parahaemolyticus, V. mimicus, V. alginolyticus,* and *Proteus rettgeri* were obtained from wild lobster, whereas *V. damsella, V. cholerae, Acinetobacter* sp., *V. mimicus, V. fluvialis* and *Enterobacter aerogenes* were discovered from farmed lobster in Telong-Elong bay, East Lombok (Lasmika, 2015). Lasmika (2015) conducted bacterial identification by using biochemical method, while in our study, we used molecular method.

In other country, in The Persian Gulf, the wild population *P. homarus* also indicated the presence of various *Vibrio* species in its hemolymph. Four *Vibrio* species had been detected were *Vibrio vulnificus, Vibrio alginolyticus, Vibrio mimicus*

Table 2. Results of bacterial pathogenicity test in lobster *Panulirus homarus* by intramuscular injection of 0.1 ml suspension of 8 bacterial isolates at density of 7 x 10^6 cfu/ml (*Hasil uji patogenisitas bakteri pada lobster Panulirus homarus dengan injeksi intramuskuler 0,1 ml masing-masing suspensi 8 isolat bakteri dengan kepadatan 7 x 10^6 cfu/ml*)

No.	Bacterial isolates (*Isolat bakteri*)		Number of lobsters (*Jumlah lobster*)	Mortality (*Mortalitas*)
1	Pa-U5-2	*Shewanella alga*	3	0
2	Pa-Hp5-1	*Bacillus firmus*	3	0
3	Pa-I3-1	*Vibrio alginolyticus*	3	0
4	L-B-U1	Unidentified-1	3	0
5	L-A-I2-4	*Tenacibaculum lutimaris*	3	0
6	L-B-I1-2	Unidentified-2	3	0
7	L-A-D1	*Pseudomonas* sp.	3	0
8	Pa-U4-1	*Vibrio* sp.	3	0
9	Injected with PBS (as negative control)		3	0

and *Vibrio harveyi.* These spesies were identified by using biochemical as well as molecular methods (Raissy *et al*., 2011; 2012).

In term of the pathogenicity test, our results demonstrated that there was no mortality of lobster recorded during five days of the test. The bacteria were possibly not pathogenic to the lobsters or might that the density of the bacteria was not adequate to induce disease infections.

In this study, *S. alga* did not causing any bacterial disease symptoms and no reports or published articles on diseases caused by this bacterium in any other mariculture species in the world. *S. alga* is known as indigenous marine bacteria, and as human pathogen causing skin and soft tissue infections (Goyal *et al*., 2011), osteomyelitis (Botelho-Nevers *et al*., 2005), abscesses, cellulitis, septicemia, gastroenteritis (bloody diarrhea) and death (Richards *et al*., 2008; Stanimirova *et al*., 2015). *S. alga* had been found in shellfish (Richards *et al*., 2008), and infecting human through raw seafood consumption or under-cooked seafood (Myung *et al*., 2009). Our results confirmed that *S. alga* could also inhabit in the mid gut of spiny lobster *P. homarus*, therefore, it is requested to remove the mid gut and avoid consuming raw or under-cooked lobsters.

The pathogenicity test showed that *Bacillus firmus* was not pathogenic to *P. homarus.* Another study also indicated that *B. firmus* did not cause either disease signs or mortalities when injected intraperitoneally or intramuscullary into *Orechromis niloticus* (Aly *et al*., 2008). The researchers stated that *B. firmus* which isolated from the stomach of *O. niloticus* exhibited potential probiotic activity. In addition, *Bacillus* spp. isolated from pylllosoma *P. ornatus* showed probiotic activity in promoting significant growth (Wietz, 2007).

Our experiment demonstrated that injection of *Vibrio alginolyticus* and *Vibrio* sp. did not caused in any mortality. This result was contradictory to the previous study by Abraham *et al.* (1996). Intramuscular injections of *V. alginolyticus* which was isolated from exoskeleton lessions and haemolymph at a level of $\geq 1.00 \times 10^8$ cells/lobster caused in 100% mortality in *P. homarus* (Abraham *et al*., 1996). This different finding might due to low concentration of the bacteria injected in this study that was only 7×10^6 cfu/ml. Vibrios are a common portion of the bacterial flora in aquaculture rearing environments and formerly considered to be predominantly opportunistic pathogens (Gomez-Gil *et al*., 1998). However, some more newly occurring

diseases in aquatic crustaceans have been affected by *Vibrio* species which perform more like true pathogens than opportunistic intruders (Lightner *et al*., 1992 in Raissy *et al*., 2011).

Further, data on the pathogenicity of *Tenacibaculum lutimaris* has not been available in other literatures, and our results also showed that there were no disease symptoms neither mortality of lobsters injected with this bacterium. *T. lutimaris* is a species from the genus *Tenacibaculum,* which previously classified as genus *Flexibacter* (Yoon *et al*., 2005). Other researcher stated that *T. lutimaris* exhibited phylogenetic relationship to *T. cellulophagum* and *T. mesophilum* (Wietz, 2007). *T. mesophilum* that isolated from pylllosoma *P. ornatus* was discovered as potential probiotic bacterium capable in clearing of a pathogenic *Vibrio harveyi* C071 in colony assay (Wietz, 2007). Bacterial isolates L-A-D1 that homologous with *Pseudomonas* sp. in our study was not caused in either mortality or bacterial disease symptoms. Other species of bacteria, *Pseudomonas perolens* which is gram-negative species is also not pathogenic to lobster *Homarus americanus* (Mori and Stewart, 2006).

In our study, the density of bacterial isolates injected for pathogencity test was considered low compared to other study, and therefore, there was no pathogenicity effects of the bacteria. It is expected that higher bacterial density would cause in different pathogenicity results and it is likely to found threshold of bacterial density which caused in mortality.

CONCLUSION

The present study identified six bacterial isolates from the wild and farmed spiny lobster *Panulirus homarus* that demonstrated high similarity of nucleotide sequence with *Shewanella algae, Bacillus firmus, Vibrio alginolyticus, Tenacibaculum lutimaris, Pseudomonas* sp. and *Vibrio* sp. Two isolates were remained unidentified as (Unidentified-1 and Unidentified-2) due to low of sequence similarity (< 97%). There were no bacterial isolates causing mortality of lobsters which were administered by intramuscular injection. It could be stated that the dose of injection (7×10^6cfu/ml) was too low to induce bacterial infections especially for *Vibrio*. Other bacteria were possibly not pathogenic to lobster, or have the potency as probiotic bacteria. We suggested further performing examination on the potency of the bacteria as probiotic for spiny lobster farming.

ACKNOWLEDGEMENT

The financial support towards this study from the Institute for Mariculture Research and Fisheries Extension (IMRAFE) funded research project of the Ministry of Marine Affairs and Fisheries, Government of Indonesia is gratefully acknowledged. We also thank Sri Suratmi for Laboratory works.

REFERENCES

Abraham, T.J., Rahman, M.K. and Joseph, M.T.L., 1996. Bacterial disease in cultured spiny lobster, *Panulirus homarus* (Linnaeus). *Journal of Aquaculture in the Tropics, 11*(3), pp. 187–192.

Aly, S.M., Abd-El-Rahman, A.M., John, G. and Mohamed, M.F., 2008. Characterization of Some Bacteria Isolated from *Oreochromis niloticus* and their Potential Use as Probiotics. *Aquaculture*, *277*(2008), pp. 1–6.

Botelho-Nevers, E., Gouriet, F., Rovery, C., Paris, P., Roux, V., Raoult, D. and Brouqui, P., 2005. First case of osteo-myelitis due to *Shewanella algae*. *Journal of Clinical Microbiology, 43*(10), pp. 5388–5390.

Gomez-Gil, B., Tron-Mayen, L., Roque, A., Turnbull, J.F., Inglis, V. and Guerra- Flores, A.L., 1998. Species of Vibrio isolated from hepatopancreas, haemolymph and digestive tract of a population of healthy juvenile *Penaeus vannamei*. *Aquaculture*, *16*(1998), pp. 1–9.

Goyal, R., Kaur, N. and Thakur, R., 2011. Human soft tissue infection by the emerging pathogen *Shewanella algae*. *The Journal of Infection in Developing Countries*, *5*(4), pp. 310–312.

Haryanti, Fahrudin, Wardana, I.K., Sembiring, S.B.M., Permana, G.N. dan Mahardika, K., 2011. Profil genotip benih udang windu *Penaeus monodon* hasil seleksi dengan karakter toleran terhadap infeksi *white spot syndrome virus*. *Jurnal Riset Akuakultur, 6*(3), pp. 393–405.

Hongoh, Y., Yuzawa, H., Ohkuma, M. and Kudo, T., 2003. Evaluation of primers and PCR conditions for the analysis of 16S rRNA genes from a natural environment. *Federation of European Micro-biological Societies, Microbiology Letters*, *221* (2), pp. 299–304.

Immanuel, G., Iyappa Raj, P., Esakki Raj, P. and Palavesam, A., 2006. Intestinal bacterial diversity in live rock lobster *Panulirus homarus* (Linnaeus) (Decapoda, Pleocyemata, Palinuridae) during transportation process. *Pan-American Journal of Aquatic Sciences*, *1* (2), pp. 69–73.

Lasmika, Ni. L. A., 2015. Study on Infectious disease in wild and cultured lobsters (*Panulirus homarus*) in West Nusa Tenggara. *Thesis*. Program Pasca Sarjana, Sekolah Tinggi Perikanan Jakarta. In Indonesian.

Leslie, V.A., Rathinam, A.M.M. and Balasingh, A., 2013. Rapid identification of *Vibrio harveyi* isolates in *Panulirus*

homarus. *International Journal of Current Microbiology and Applied Science*, *2*(3), pp. 6–10.

Maiti, B., Shekar, M., Khushiramani, R., Karunasagar, I. and Karunasagar, I., 2009. Evaluation of RAPD-PCR and protein profile analysis to differentiate *Vibrio harveyi* strains prevalent along the southwest coast of India. *Journal of Genetics*, *88*(3), pp. 273–279.

Mori, K. and Stewart, J.E., 2006. Immunogen-dependent quantitative and qualitative differences in phagocytic responses of the circulating hemocytes of the lobster *Homarus americanus. Disease of Aquatic Organism*, *69*, pp. 197–203.

Myung, D.S., Jung, Y., Kang, S., Song, Y.A., Park, K., Jung, S., Kim, S.H. and Shin, J., 2009. Primary *Shewanella algae* Bacteremia Mimicking *Vibrio Septicemia. Journal of Korean Medical Science*, *24*(6), pp. 1192–1194.

Payne, M.S., Hall, M.R., Sly, L. and Bourne, D.G., 2007. Microbial Diversity within Early-Stage Cultured *Panulirus ornatus* Phyllosomas. *Applied and Environmental Microbiology*, *73*(6), pp. 1940–1951.

Raissy, M., Momtaz, H., Moumeni, M., Ansari, M. and Rahimi, E., 2011. Molecular detection of *Vibrio* spp. in lobster hemolymph. *African Journal of Microbiology Research*, *5*(13), pp. 1697–1700.

Raissy, M., Moumeni, M., Ansari, M. and Rahimi, E., 2012. Occurrence of *Vibrio* spp. in lobster and crab from the Persian Gulf. *Journal of Food Safety*, *32*(2), pp. 198–203.

Richards, G.P., Watson, M.A., Crane III, E.J., Burt, I.G. and Bushek, D., 2008. *Shewanella* and *Photobacterium* spp. in oysters and seawater from the Delaware Bay. *Applied Environmental Biology*, *74*(11), pp. 3323–3327.

Rungrassamee, W., Klanchui, A., Maibunkaew, S., Chaiyapechara, S., Jiravanichpaisal, P. and Karoonuthaisiri, N., 2014. Characterization of intestinal bacteria in wild and domesticated adult black tiger shrimp (*Penaeus monodon*). *PLoS ONE, 9* (3), pp. 1–11.

Stanimirova, I., Petrova, A. and Murdjeva, M., 2015. Case of *Shewanella putrefaciens* Gastroenteritis in Bulgaria – an Evaluation of *Shewanella* Role in Infectious Diarrhea. *Sikkim Manipal University (SMU) Medical Journal*, *2*(2), pp. 215–227.

Wietz, M., 2007. Microbial communities of larval rearing systems and wild phyllosoma of the ornate rock lobster, *Panulirus ornatus*, and options for microbial management in aquaculture. *Diploma Thesis*. Faculty of Biology, University of Bremen, Germany.

Yoon, J., Kang, S. and Oh, T., 2005. *Tenacibaculum lutimaris* sp. nov., isolated from a tidal flat in the Yellow Sea, Korea. *International Journal of Systematic and Evolutionary Microbiology, 55*(2), pp. 793–798.

Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput harus menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up to date*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan atau baru, agar dapat segera diketahui oleh umum. Hasil dan pembahasan dapat digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah Bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul diberikan dalam bahasa Indonesia dan inggris. Judul ditulis dalam huruf tegak kecuali untuk nama ilmiah yang menggunakan bahasa latin, Judul harus singkat, jelas dan mencerminkan isi naskah dengan diikuti oleh nama serta alamat surat menyurat penulis dan alamat email. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*). Jika penulis lebih dari satu orang bagi pejabat fungsional penelitian, pengembangan agar menentukan status sebagai kontributor utama melalui penandaan simbol dan keterangan sebagai kontributor utama dicatatan kaki di halaman pertama artikel.
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam Bahasa Inggris merupakan terjemahan dari Bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Perlu disebutkan juga studi terdahulu yang pernah dilakukan terkait dengan penelitian yang dilakukan.
5. **Bahan dan cara kerja**
   Bahan dan cara kerja berisi informasi mengenai metode yang digunakan dalam penelitian. Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasinya dan apabila ada modifikasi maka harus dituliskan dengan jelas bagian mana dan hal apa yang dimodifikasi.
6. **Hasil**
   Hasil memuat data ataupun informasi utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada suatu tabel/ grafik/diagram atau gambar, maka hasil yang terdapat pada bagian tersebut dapat diuraikan dengan jelas dengan tidak menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata- rata maka harus menyertakan pula standar deviasinya.
7. **Pembahasan**
   Pembahasan bukan merupakan pengulangan dari hasil. Pembahasan mengungkap alasan didapatkannya hasil dan arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, hasil penelitian ini dapat dibandingkan dengan studi terdahulu.
8. **Kesimpulan**
   Kesimpulan berisi infomasi yang menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, implikasi dari hasil penelitian dan penelitian berikutnya yang bisa dilakukan.
9. **Ucapan terima kasih**
   Bagian ini berisi ucapan terima kasih kepada suatu instansi jika penelitian ini didanai atau didukungan oleh instansi tersebut, ataupun kepada pihak yang membantu langsung penelitian atau penulisan artikel ini.
10. **Daftar pustaka**
    Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review*. Apabila harus menyitir dari "laporan" atau "komunikasi personal" dituliskan '*unpublished*' dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers* dan penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Microsoft Word, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak spasi tunggal. Batas kiri-kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan Bahasa Indonesia, angka desimal ditulis dengan menggunakan koma (,) dan ditulis dengan menggunakan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5 cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi ditulis dengan merujuk kepada aturan standar yang diakui. Untuk tumbuhan menggunakan *International Code of Botanical Nomenclature* (ICBN), untuk hewan menggunakan International *Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae*, *Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
6. Untuk range angka menggunakan en dash (–), contohnya pp.1565–1569, jumlah anakan berkisar 7–8 ekor. Untuk penggabungan kata menggunakan hyphen (-), contohnya: masing-masing.
7. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
8. Tabel
   Tabel diberi judul yang singkat dan jelas, spasi tunggal dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah.

8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul gambar ditulis secara singkat dan jelas, spasi tunggal. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi, untuk *line drawing* minimal 600dpi.
9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al*. Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al*., 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995). Jika sitasi beruntun maka dimulai dari tahun yang paling tua, jika tahun sama maka dari nama penulis sesuai urutan abjad. Contoh: (Anderson, 2000; Agusta *et al*., 2005; Danar, 2005). Penulisan daftar pustaka, sebagai berikut:

   a. **Jurnal**
      Nama jurnal ditulis lengkap.
      Agusta, A., Maehara, S., Ohashi, K., Simanjuntak, P. and Shibuya, H., 2005. Stereoselective oxidation at C-4 of flavans by the endophytic fungus *Diaporthe* sp. isolated from a tea plant. *Chemical and Pharmaceutical Bulletin*, *53*(12), pp.1565–1569.
   b. **Buku**
      Anderson, R.C. 2000. *Nematode Parasites of Vertebrates, Their Development and Tramsmission*. 2nd ed. CABI Publishing. New York. pp. 650.
   c. **Prosiding atau hasil Simposium/Seminar/Lokakarya.**
      Kurata, H., El-Samad, H., Yi, T.M., Khammash, M. and Doyle, J., 2001. Feedback Regulation of the Heat Shock Response in *Eschericia coli*. *Proceedings of the 40th IEEE Conference on Decision and Control*. Orlando, USA. pp. 837–842.
   d. **Makalah sebagai bagian dari buku**
      Sausan, D., 2014. Keanekaragaman Jamur di Hutan Kabungolor, Tau Lumbis Kabupaten Nunukan, Kalimanan Utara. Dalam: Irham, M. & Dewi, K. eds. *Keanekaraman Hayati di Beranda Negeri*. pp. 47–58. PT. Eaststar Adhi Citra. Jakarta.
   e. **Thesis, skripsi dan disertasi**
      Sundari, S., 2012. Soil Respiration and Dissolved Organic Carbon Efflux in Tropical Peatlands. *Dissertation*. Graduate School of Agriculture. Hokkaido University. Sapporo. Japan.
   f. **Artikel online.**
      Artikel yang diunduh secara online ditulis dengan mengikuti format yang berlaku untuk jurnal, buku ataupun thesis dengan dilengkapi alamat situs dan waktu mengunduh. Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses peer review misalnya laporan perjalanan maupun artikel dari laman web yang tidak bisa dipertangung jawabkan kebenarannya seperti wikipedia.
      Himman, L.M., 2002. A Moral Change: Business Ethics After Enron. San Diego University Publication. *http:ethics.sandiego.edu/LMH/oped/Enron/index.asp*. (accessed 27 Januari 2008) bila naskah ditulis dalam bahasa inggris atau (diakses 27 Januari 2008) bila naskah ditulis dalam bahasa indonesia

Formulir persetujuan hak alih terbit dan keaslian naskah
Setiap penulis yang mengajukan naskahnya ke redaksi Berita Biologi akan diminta untuk menandatangani lembar persetujuan yang berisi hak alih terbit naskah termasuk hak untuk memperbanyak artikel dalam berbagai bentuk kepada penerbit Berita Biologi. Sedangkan penulis tetap berhak untuk menyebarkan edisi cetak dan elektronik untuk kepentingan penelitian dan pendidikan. Formulir itu juga berisi pernyataan keaslian naskah yang menyebutkan bahwa naskah adalah hasil penelitian asli, belum pernah dan tidak sedang diterbitkan di tempat lain serta bebas dari konflik kepentingan.

Penelitian yang melibatkan hewan
Setiap naskah yang penelitiannya melibatkan hewan (terutama mamalia) sebagai obyek percobaan/penelitian, wajib menyertakan *'ethical clearance approval'* terkait animal *welfare* yang dikeluarkan oleh badan atau pihak berwenang.

Lembar ilustrasi sampul
Gambar ilustrasi yang terdapat di sampul jurnal Berita Biologi berasal dari salah satu naskah yang dipublikasi pada edisi tersebut. Oleh karena itu, setiap naskah yang ada ilustrasinya diharapkan dapat mengirimkan ilustrasi atau foto dengan kualitas gambar yang baik dengan disertai keterangan singkat ilustrasi atau foto dan nama pembuat ilustrasi atau pembuat foto.

Proofs
Naskah *proofs* akan dikirim ke penulis dan penulis diwajibkan untuk membaca dan memeriksa kembali isi naskah dengan teliti. Naskah proofs harus dikirim kembali ke redaksi dalam waktu tiga hari kerja.

Naskah cetak
Setiap penulis yang naskahnya diterbitkan akan diberikan 1 eksemplar majalah Berita Biologi dan *reprint*. Majalah tersebut akan dikirimkan kepada *corresponding author*

Pengiriman naskah
Naskah dikirim secara online ke website berita biologi: *http://e-journal.biologi.lipi.go.id/index.php/berita_biologi*

Alamat kontak
Redaksi Jurnal Berita Biologi, Pusat Penelitian Biologi-LIPI
Cibinong Science Centre, Jl. Raya Bogor Km. 46 Cibinong 16911
Telp: +61-21-8765067, Fax: +62-21-87907612, 8765063, 8765066,
Email: berita.biologi@mail.lipi.go.id
jurnalberitabiologi@yahoo.co.id atau
jurnalberitabiologi@gmail.com

BERITA BIOLOGI

Vol. 18(3) **Isi (*Content*)** Desember 2019

P-ISSN 0126-1754
E-ISSN 2337-8751

MAKALAH HASIL RISET (*ORIGINAL PAPERS*)

KOMUNIKASI PENDEK (*SHORT COMMUNICATION*)