Proses Peer review Artikel pada Jurnal Biodiversitas

Scren shot pada layar OJS Jurnal

Revised paper

Participants

Ayu Astuti (ayu)

Rasheed Ahmad (rasheed2)

Messages

Note	From
Kindly inform us your revised paper	ayu 2021-02-09 02:07 PM
We just send our revised paper and also answers for the reviewer's comments in other documents. Thank you. rasheed2, Revision_Identification of marine sponges-symbiotic bacteria and their application in degrading polycyclic aromatic hydroc.docx rasheed2, Reviewer's Comments.docx	rasheed2 2021-02-22 03:38 AM

Komentar dan respone Penulis atas koreksi reviewers “J” dan “S” (Round -1)
(Highlight Kuning merupakan respon penulis)

From: **Dr. Rasheed Ahmad** <rasheed.ahmad@fst.unair.ac.id>
Date: Fri, 8 Jan 2021 at 13:11
Subject: Re: [biodiv] Editor Decision
To: <mudya07@gmail.com>

Please see the response of the journal attached.

On Thu, Jan 7, 2021 at 11:55 AM Smujo Editors <smujo.id@gmail.com> wrote:

Rasheed Ahmad:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Identification of marine sponges-symbiotic bacteria, and their application in degrading polycyclic aromatic hydrocarbons".

Our decision is: Revisions Required

Note: Kindly send your revised paper to professional English proofreader prior to resubmission. Table of Response is olso needed.

Smujo Editors
editors@smujo.id

Reviewer J:

1. In abstract, Please rewrite the following sentences "Diverse and abundant microbial species.. .... are related symbiotically".

2. Please mention the colony-forming unit of bacterial suspension which was used as an inoculum.

3. English can be improve.

Recommendation: Revisions Required

Reviewer S:

Review: Identification of marine sponges-symbiotic bacteria, and their application in degrading polycyclic aromatic hydrocarbons

The goal of the study was to isolate and characterize sponge-associated bacteria and to examine their ability to degrade polycyclic aromatic hydrocarbons (PAHs) naphthalene and pyrene. From the four sponges, 14 bacterial isolates were isolated, of which eight were further identified and characterized. Isolates from the same source tend to share similar morphological features. The authors reported that the isolates SpCB1 and SpCB2 can degrade naphthalene whereas isolate SpDB1 can degrade both naphthalene and pyrene. This is a potentially interesting field of research. However, the manuscript as written requires some major improvements.

In particular, there was insufficient background and justification for the experimental design. Moreover, the choice of methods was not explained nor described in sufficient detail, experimental controls were not included, and no statistical analysis was done to differentiate responses. Thus, claims in the discussion and conclusion are inaccurately stated and are not strongly supported by the results. For example, the inoculum density for the PAH degradation experiment was not standardized. Hence, differences in cell count may influence degradation activity. Also, there was no basis in choosing the PAH type and concentrations used. It might have been better to perform a preliminary experiment or include a dose-response analysis to reveal optimal degradation activities. Furthermore, the parameters measured are not the best proxies for measuring PAH degradation. While optical density, pH, air bubble, and fermented flavor represent growth and utilization of PAHs in the absence of other carbon sources, changes in the concentration of PAHs and their intermediates should also have been measured. Alternatively, the authors could restate their conclusions to simply indicate that their isolates are able to grow on two PAH types as their sole source of carbon, thereby implying that they can degrade these molecules. A statistical analysis to compare growth rates of the different isolates would also be useful to pinpoint ones with greater potential for bioremediation applications. Limitations of the study should be very clearly acknowledged in discussion/conclusion sections.

For replicability and for future bioremediation applications, it is also important to determine identity of the sponge source from which the bacteria were isolated. It is recommended that morphological characterization of the sponge host or marker gene analysis be conducted to verify sponge identities. The phylogenetic reconstruction of the 16S sequences of the isolates needs to be reviewed, taking into consideration an appropriate substitution model and alternative phylogenetic approaches aside from neighbor joining method.

Major editing for language and writing quality are needed to clearly present the results and to efficiently relate the findings to other studies.

Specific comments:

Title: Remove comma after bacteria

Abstract:

Line 14: Indicate species of sponge hosts that were the source of the microbes.

1. *Neopetrosia* sp; 2. *Aulleta* sp 3. *Petrosia (Strongylophora) corticata dan 4. Hyrtios erectus*

Lines 14-16: Can be combined into one concise sentence. Add a short statement on the implications of the study in the last sentence of the abstract

Bacillus and Pseudomonas bacteria isolated from hydrocarbon contaminated sponges can degrade Naphthalene and pyrene PAHs.

Keywords:

Line 23: Marine sponges symbiont bacteria > sponge-associated bacteria (ok)
Line 24: Instead of ribosomal DNA (rDNA), use the term ribosomal RNA gene (rRNA) consistently throughout the manuscript (ok)

Introduction: Rationale and objectives should be clearly stated in this section

Line 28: host > hosts (ok)

Lines 28-29: Reference? (ok)

Line 31: Pori bacteria -> Poribacteria (ok)

Line 50: Remove comma after (2014) (ok)

Materials and methods: Methods should be clear and detailed so as to enable other researchers to interpret and replicate your results.

Line 59-60: Please comment why sponges are collected in these sites? Are the selected sites impacted or threatened by coastal run-offs?

The sponge sampling location was chosen because that location is a Makassar Marine Tourism development area and is also very close to transportation routes for various types of ships, meaning that in this area there is a lot of interaction with humans, so the potential for PAH contamination of biota (sponges) is relatively high, on the other hand the area is it is part of a conservation area that is included in the Spermonde archipelago

Line 59: Do you have in situ pictures of the sponges? Did you try to identify the collected sponges based on morphological/anatomical features or marker genes? What is the basis of collecting the target sponge species? What is the collection depth?

Side depth between 4.2 m – 4.8 m. Type of sponge based on the results of morphological analysis: SpA. (Neopetrosia sp); SpB (Aulleta sp) and SpC (Petrosia (Strongylophora) corticata) and SpD (Hyrtios erectus) (Marzuki et al., 2020) recent additional references

Line 61-62: Delete "We isolated fourteen bacterial isolates from four types of sponges that collected" since the method for isolating sponge associated bacteria can be found in line70-79.

Obviously just delete it

Line 66-69: The figure of the sampling site is not readable and needs to be at higher resolution.

Revised. See lines 66-68

Line 74-75: Add the total volume of the PBS buffer. What was the volume of inoculum that was spread into the SWC media?

Total PBS Volume 5 mL. The volume of inoculum spread on SWC media was ± 1 mL

Line 86: specify what "typical biochemical tests" were conducted

(there are 15, parameters according to table 3)

Line 92-93: Since the primer sequences are already mentioned here, there is no need to add Table 2 unless multiple primers were used to amplify the 16S rRNA gene.

(It is clear that table 2 has been deleted). If it is deleted, it means that tables 3 – 6 are changed to tables 2-5 (I have changed it in the manuscript, we just add the reference in line: between 93-95

Line 93-94: Add the expected size of the PCR amplicons and the thermal cycler conditions.

(Nitrogen base pairs with size adjusted primers (revised)

Line 94: Please add the correct PCR kit used to amplify the 16S rRNA gene. SYNC DNA Extraction Kit is used to extract genomic DNA from environmental samples or tissues and not for PCR.

Extraction of 16 rRNA gene DNA:
ddH2O, chelex 20%, a pair of universal primary sequences of the 16S rRNA gene of E. coli: FP-U1 (5'-CCAGCAGCCGCGGTAATACG-3') at nucleotides 518-537, and RP-U2 is (5'- ATCGG (C/T) TACCTTGTTACGACTTC-3') corresponding to nucleotides 1513-1491, template DNA, Taq DNA polymerase (Perkin- Elmer, Norwalk, Conn), PCR Mix, Triton X-100, Tris, EDTA, HCl, KCl, $MgCl_2$, paraffin, deoxynucleoside triphosphate, polyacrylamide gel, agarose, Ethidium Bromide.

Line 94: "ejected" should be "excised" (ok)

Line 96: Add the final volume of the PCR product and the primers used for direct sequencing.

The membrane was transferred to sterile tubes separately and the cells were dissolved in the bead beater. Bacterial DNA extraction was carried out using the AxyPrep™ Bacterial Genomic DNA Miniprep Kit (Axygen Biosciences, Inc., CA, USA) extraction kit. Total DNA of extracted symbionts was amplified with universal primers of 16S rRNA *E. coli*, namely forward primer FP-U1 (5'-CCAGCAGCCGCGGTAATACG-3') attached to the nucleotide base of 518-537, and reverse primer RP-U2 (5' -ATCGG (C/T) TACCTTGTTACGACTTC-3'), attached to the nucleotide base sequence 1.513-1.491. The total volume of PCR mix was 30 µL containing 19 µL DreamTaq buffer (Fermentas, MA), 0.2 µL dNTP (dATP, dTTP, dGTP, dCTP), 0.05 lL units, 1 DreamTaq polymerase (Fermentas), 1 µL dNTP (dATP, dTTP, dGTP, dCTP), 0.05 lL units, 1 DreamTaq polymerase (Fermentas), 1 µL forward and reverse primers. The PCR amplification process was carried out in 30 cycles at 95°C for 5 min, denaturation at 94°C for 1 min, annealing at 54°C for 1 min 20 seconds, extension at 72°C for 2 min, and elongation at 72°C for 10 min.

Line 101: 6 references sequences? Or 9? Please check. Add more 16S sequences for phylogenetic analysis for broader taxon representation. The alignment should also be presented in supplemental materials, if possible. (has been revised)

Line 102: DNA GeneBank of NCBI > NCBI GenBank (has been revised)

Line 107-114: Clarify how you standardized the amount of bacterial cells used the biodegradation activity experiment. How many cells were used per tube? Clarify how you measured each of the parameters shown in Table 5 and 6 (What is fermentation flavor? What is activity?).

1. The number of bacterial cells used in each vial (tube) is in the range of 2.62.104 cells/mL – 3.45. 104 cells/mL.
2. The standard used is the optical density (turbidity) of the media at zero day of interaction between the bacterial suspension and PAHs (anthracene/pyrene)
3. Air bubbles or gas, the smell of fermentation are characteristic of the fermentation reaction as an indicator that degradation of the PAH (anthracene/pyrene) molecular structure has occurred which results in degradation products in the form of simple organic compounds and CO_2 gas. Visual observations were made, while the media used to be more acidic by measuring the pH of the media using universal pH indicator paper

4. To ascertain the types of simple organic compounds that are products of bacterial biodegradation of anthracene/pyrene, they should be measured in the media using GC-MS so that the abundance of PAHs is obtained whether it decreases or remains and can also determine the types of organic compounds resulting from biodegradation.
5. Point 4 is a limitation of this publication in the next publication

Line 110: Eppendorf and Falcon are both brand names for tubes. Better to indicate size and type of tube (e.g. 50ml conical Falcon tubes)

It has been revised, to be precise in line 112 (there is a shift from adding libraries that are not included in the Mendelay system

Line 111-113: How many replicates did you have? What were your controls?

1. Replications were carried out 3 times, each measurement was carried out in 3 vials (tubes) in parallel and the same treatment.
2. Contral is the optical density (turbidity) at zero day of interaction (see table 4 after adjusting the serial number of the table)

Line 115-121: You can omit the ‘Data analysis’ section since these methods are already mentioned previously.

(It is clear whether it can be lost or not, if we keep this part, maybe the sentence needs to be strengthened so that there is a difference with the previous sentences)

Line 122: Table 1 should be Table 2.

(ok) it's been adjusted what is meant by the primary table which is only 1 row) because it was mentioned before.

Results:

Line 123: Results and discussion should only be Results because the authors have a separate discussion section. (has been revised)

Lines 124-147: Should be combined into one paragraph

(has been revisid)

Line 125: Clarify if the 14 bacteria were isolated from the sponge surface or from the mesohyl.

(All 14 bacteria were isolated from the mesohyl of 4 types of sponges, namely [SpA. (Neopetrosia sp); SpB (Aulleta sp) and SpC (Petrosia (Strongylophora) corticata) and SpD (Hyrtios erectus) (Marzuki et al., 2020) reference recent addition.] see table 1, sponge sample code

Line 126: Remove “(Figure 1)”

(ok) has been deleted in the manuscript

Line 126-130: Delete sponge collection sites since these are already presented in methods.

Coordinate point on line 133 -137. Already in table 1, has been revised.

Line 131-133: Adding more reference 16S sequences in the phylogenetic analysis might help determine proper taxonomic placement in the tree.

(Explanations have been developed

Line 133: Revise "have been completely determined their DNA sequencing and identified phylogenetically" to "have been identified through direct sequencing of the 16S rRNA gene."

Sentences have been corrected

Line 135: See comment on identifying the collected sponges in methodology.

(The sentence in question has been corrected)

Lines 155-157: Mention that the sequences of the isolates clustered to the available sequences of Bacillus and Pseudomonas species.

(bacillus group species for bacterial isolates coded SpAB1; SpAB2; and SpBB1; SpBB2) and pseudomonas group species for bacterial isolates coded SpCB1; SpCB2 and SpDB1; SpDB2)

Line 156: Revise "Among total 14 sponge symbiont bacteria isolate in this study, only 6 reference sequence results (948 – 955 bp) of the 16S rRNA region identified phylogenetically" to "Among the 14 sponge symbiotic bacteria isolated in this study, only 6 bacterial isolates (948 – 955 bp) were identified phylogenetically."

((The sentence in question has been corrected)

Line 161-166: Please comment on why only 8 bacterial isolated were tested for biodegradation activity when you had 14 bacteria isolates from the sponges.

Only 8 bacteria continued to test the biodegradation activity of anthresene and pyrene. The determination of these bacteria is based on the results of biochemical tests, in which bacteria that have the potential to degrade carbon compounds are bacteria that have a minimal positive reaction with reagent 1. Red Methyl; 2. Voges-Proskauer; 3. Citric; 4. Lactose and 5. Reagent Indole. The other 6 sponge symbiotic bacteria did not show a positive reaction to the above reagent (see table 3). Thus the other 6 types of bacteria were not selected as biodegraders in the biodegradation activity test on the tested hydrocarbons (anthracene and pyrene).

Line 181-183: Are the 4 sponges collected in this study of different species? It is important to identity the host sponge in order to further understand the potential ecological and biotechnological application of the bacterial isolates.

The 4 identified sponges came from 4 different species, namely the sponge species with sample code SpA= (Neopetrosia sp); SpB = (Aulleta sp) SpC = (Petrosia (Strongylophora) corticata) and SpD = (Hyrtios erectus) (Marzuki et al., 2020c) recent additional references. Sponge species were determined using the morphological analysis method that had been carried out previously (Marzuki et al., 2020c)

Line 195-197: Care should be taken in interpreting or correlating the result of the biochemical tests to the PAH biodegradation activity of the isolates.

Biochemical tests were carried out to see and select potential bacteria in the biodegradation of hydrocarbons, especially anthrazen and pyrene PAHs which were based on positive reaction results against 5 types of biochemical reagents, namely: 1. Red Methyl; 2. Voges-Proskauer; 3. Citric; 4. Lactose and 5. Reagent Indole

Discussion: Implications and limitations of the study should be clearly stated in this section. Link your findings to what has been reported in other studies.

Lines 198-202: Should be transferred to results (identification and phylogenetic analysis of sponge symbiont bacteria)

(About pylogenic, this section has been added and revised)

Line 203-205: Care should be taken in interpreting or correlating the result of the biochemical tests to the PAH biodegradation activity of the isolates.

The selection of bacterial species for the bioactivity test for degradation of anthracene and pyrene was based on the results of a positive reaction to 5 theagens in the biochemical test (Table 3). The selected bacteria were shown to have bioactivity in degradation of anthracene and pyrene PAHs (Tables 4 and 5). The positive reaction of bacteria to 5 types of biochemical reagents provides information that these bacteria: 1). can carry out fermentation reactions, 2) these bacteria have the ability to make carbon as a nutrient and energy source, and 3) these bacteria have the ability to destroy the structure of carbon compounds to produce simple organic compounds. The identification of the markers of anthracene and pyrene biodegradation by bacteria is the appearance of a fermentation odor, the presence of CO_2 gas bubbles and an increase in the acidity of the degradation medium.

Source text Source text is required to get additional translation information

Send feedback

Side panel

Line 207-210: See Esteves (2016) Sample Processing Impacts the Viability and Cultivability of the Sponge Microbiome.

(Already included Esteves references in the reference list, but I have not included any comments related to the viability of bacteria in the media) please add around Line 207 – 217)

Line 213: napthalena > naphthalene

(ok) has been adjusted in the manuscript

Line 212-214: Please comment if the selected sites are affected by coastal run-off. What are the ecological applications of the ability of sponge-associated bacteria to degrade PAHs?

The sampling locations were 4 out of 5 islands in the Makassar marine tourism area, where these islands are also part of the Spermonde Archipelago cluster which harbors abundant populations of various types of sponges. This area is also included in the transportation routes of various ships where the area has been identified as being exposed to hazardous waste in the form of heavy metals and hydrocarbons (Marzuki et al 2020b), but currently we can still find several types of sponges that are able to survive. It was found that there were red threads of a sponge with a body surface covered in mucus and the ability of symbiont bacteria to degrade PAHs (Marzuki, 2018). Sponge exploration book. The influence of undersea currents and coastal wave overtopping is also thought to have an influence on the ability of sponges to adapt to the dynamics of ocean pollution, currents and waves. The ability of sponges to produce mucus to cover their body surface is thought to be a form of adaptation of sponges to environmental conditions. Therefore it should be suspected that sponges and symbiotic bacteria that become sponges as hosts can also overcome the toxicity of PAHs. Isolation of bacteria from the Mesohyl sponge, not from the surface of the sponge body, because it is considered that bacteria that live on the surface of the sponge body can certainly adapt to their environment (Marzuki et al., 2015a).

Line 236-238: Care should be taken in interpreting or correlating sponge morphology, the result of the biochemical tests, and the biodegradation activity of the isolates.

Based on the results of previous studies, it is suspected that there is a correlation between the morphology of sponges and the production of mucus on their body surface as a form of self-protection against various predators. The biochemical test shows the specification that if the bacteria react positively to the 5 types of biochemical reagents as described above, it means that these bacteria have the ability to carry out fermentation reactions, making carbon a nutrient an energy source and can destroy hydrocarbon molecules. The biodegradation activity of this sponge isolate towards anthracene and pyrene is a manifestation of the ability of sponges and bacteria to adapt to their environment.

Figures: Figures are blurry.

Images have been upscaled, although the results are not satisfactory.

Figure 3: Indicate standard deviations for each point; statistical analysis is needed

It seems that the standard deviation does not need to be shown in numbers, because there is already data info from the regression equation and R2.

Tables:

Lines 80: Tabel 1 > Table 1

(ok) has been corrected in the manuscript

Line 122: Table 1 (that should be Table 2) is not necessary. Remove.

(There was an error in writing the table number because a table was deleted. It has been revised)

Table 3: SpAB1 and SpAB2 have similar characteristics. Just show only one set of characters for the two isolates.

SPAB1 and SpAB2 do not have a precise reaction character against biochemical reagents, thus the characters that can be shown in the degradation bioactivity test against the tested PAHs (anthracene and pyrene) are also expected to be different. This is evidenced by the difference in degradation bioactivity shown by the two (see table 4). and 5).

Line 149: Note below the table is not necessary. Remove. It is already indicated in the last column of the table.

(Table 2 with Note: (-) : Gram – (Negative Bacteria) and (+) : Gram-Positive Bacteria; ask to be deleted

References:

Line 245: Omit ADDIN Mendeley Bibliography CSL BIBLIOGRAPHY

Has been revised

Recommendation: -