Rika Pratiwi <pratiwi@unika.ac.id>

Fwd: Submission of Full Paper and Post-event Informastion

1 message

DYAH WULANDARI <dyahwulandari@unika.ac.id> Sun, Apr 21, 2024 at 5:12 AM
To: Rika Pratiwi <pratiwi@unika.ac.id>

---------- Forwarded message ---------
From: **CCRMS IPB** <ccrms_conference@apps.ipb.ac.id>
Date: Tue, Sep 19, 2023, 21:43
Subject: Submission of Full Paper and Post-event Informastion
To: <wahyu.ramadhan@apps.ipb.ac.id>

Dear Esteemed Participants,

We would like to express our sincere gratitude for your presence and enthusiasm during The 4th International Conference on ICMMBT that took place a few days ago.

We are pleased to provide you with some essential post-event information, including:

1. Deadline for Full Paper Submission: September 25, 2023 You can submit your full paper using the following links:
Template/Guidelines for Paper Writing Full Paper Guidelines https://ipb.link/full-paper-guideline-icmmbt2023
Full Paper Submission: Submit Full Paper https://ipb.link/fullpapericmmbt

2. Keynote Speaker Presentation Materials: Access the PowerPoint presentations from our keynote speakers through this link:
Keynote Speaker Materials and Abstracts https://ipb.link/material-icmmbt

3. Event Documentation: You can view the documentation of the conference proceedings by visiting this link:
ICMMBT Event Documentation https://ipb.link/doc-icmmbt

4. And lastly, Regarding the participant certificates, we will promptly send them as soon as possible.

We hope that the information provided will be of great assistance to you. We are also open to receiving any questions or feedback you may have. Your input is valuable to us.

Thank you for your attention and participation.

Warm regards,

ICMMBT Committee

Unika SOEGIJAPRANATA

Rika Pratiwi <pratiwi@unika.ac.id>

Fwd: Review of manuscript (R1) ICMMBT 2023

1 message

DYAH WULANDARI <dyahwulandari@unika.ac.id> Sun, Apr 21, 2024 at 2:42 PM
To: Rika Pratiwi <pratiwi@unika.ac.id>

---------- Forwarded message ---------
From: **CCRMS IPB** <ccrms_conference@apps.ipb.ac.id>
Date: Sat, Nov 4, 2023, 09:24
Subject: Review of manuscript (R1) ICMMBT 2023
To: <anto.budiharjo@live.undip.ac.id>, <dyahwulandari@unika.ac.id>

Dear Author(s),

Please find the attached files from the reviewer. Kindly respond to the reviewer comments' and make the necessary revisions to the manuscript as suggested by the reviewer.

Please send back all revised documents along with the rebuttal letter to this email by **20 November 2023.**

Best regards,
ICMMBT committee

2 attachments

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Rika Pratiwi <pratiwi@unika.ac.id>

Fwd: Urgent: Submission Finalization for Web Bios Conference - Action Required by February 10, 2024

3 messages

DYAH WULANDARI <dyahwulandari@unika.ac.id> Sun, Apr 21, 2024 at 5:11 AM
To: Rika Pratiwi <pratiwi@unika.ac.id>

---------- Forwarded message ---------
From: **CCRMS IPB** <ccrms_conference@apps.ipb.ac.id>
Date: Wed, Feb 7, 2024, 20:40
Subject: Urgent: Submission Finalization for Web Bios Conference - Action Required by February 10, 2024
To: CCRMS IPB <ccrms_conference@apps.ipb.ac.id>

Dear Author(s),

We are currently in the final stages of preparing to submit the last step of the manuscript to the Web Bios conference platform.
To address the requirements, we need your agreement and the transfer of copyright to the publisher.

Kindly complete the attached file, sign it (signed by at least one author), and return the signed document to us in PDF format by **February 10, 2024**.

Best regards,
ICMMBT 2023

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DYAH WULANDARI <dyahwulandari@unika.ac.id> Sun, Apr 21, 2024 at 5:12 AM
To: Rika Pratiwi <pratiwi@unika.ac.id>

---------- Forwarded message ---------
From: **DYAH WULANDARI** <dyahwulandari@unika.ac.id>
Date: Thu, Feb 8, 2024, 12:56
Subject: Re: Urgent: Submission Finalization for Web Bios Conference - Action Required by February 10, 2024
To: CCRMS IPB <ccrms_conference@apps.ipb.ac.id>, <kustiaz@apps.ipb.ac.id>
Cc: Anto Budiharjo <anto.budiharjo@live.undip.ac.id>

Dear Editor,

Attached is the revision of the manuscript,

Thank you

Best Regards,

On Wed, Feb 7, 2024 at 8:40 PM CCRMS IPB <ccrms_conference@apps.ipb.ac.id> wrote:

> Dear Author(s),
>
> We are currently in the final stages of preparing to submit the last step of the manuscript to the Web Bios conference platform.
> To address the requirements, we need your agreement and the transfer of copyright to the publisher.
>
> Kindly complete the attached file, sign it (signed by at least one author), and return the signed document to us in PDF format by **February 10, 2024**.
>
> Best regards,
> ICMMBT 2023

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Dyah Wulandari, Ph.D

- Secretary of Food Technology Department,
- Head of Molecular and Food Biotechnology Laboratory, Food Technology Department

- International Affairs of Food Technology Department

Faculty of Agricultural Technology - Soegijapranata Catholic University (SCU), UNIKA Semarang
Jl. Rm. Hadisoebeno Sosro Wardoyo, Jatibarang, Kec. Mijen, Kota Semarang, Jawa Tengah, 50219, INDONESIA. https://foodtech.unika.ac.id/ .
email: dyahwulandari@unika.ac.id.
phone: +6281283282013

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DYAH WULANDARI <dyahwulandari@unika.ac.id> Sun, Apr 21, 2024 at 5:12 AM
To: Rika Pratiwi <pratiwi@unika.ac.id>

---------- Forwarded message ---------
From: **DYAH WULANDARI** <dyahwulandari@unika.ac.id>

Date: Sun, Feb 11, 2024, 07:25
Subject: Re: Urgent: Submission Finalization for Web Bios Conference - Action Required by February 10, 2024
To: CCRMS IPB <ccrms_conference@apps.ipb.ac.id>

Dear CCRMS Editorial Team,

Attached is the License Agreement of our manuscript.

Thank you

Best Regards,
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Antibacterial activity and molecular identification of soft coral *Sinularia* sp. symbiontic bacteria from Karimunjawa Island against skin pathogens *Propionibacterium acnes* and *Staphylococcus epidermidis*

Abstract. Soft corals can produce bioactive compounds that act as antibacterials. *Sinularia* sp. is a soft coral that can produce antibacterial compounds. Symbion bacteria are bacteria that can produce bioactive compounds that tend to be the similar or same as their host. The use of symbiont bacteria can be an alternative to exploit the potential of *Sinularia* sp. without harming marine life. This study aims to determine the antibacterial potential of the symbiont bacteria obtained from the soft coral *Sinularia* sp. in inhibiting the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis*. The antibacterial activity test was carried out by the agar diffusion method using disc paper. Antibacterial activity was indicated by the formation of a clear zone around the disc paper disc. The results showed that LA7 isolate had the best antibacterial activity with an average clear zone size of 14.6 mm against *Staphylococcus epidermidis* and 16.6 mm against *Propionibacterium acnes*. LA7 isolates was identified species based on the 16S rRNA gene with primers 27F and 1492R. Based on molecular species support, it was found that LA7 isolate is *Bacillus aerius* with a homology level of 98.17%.

Keywords: Acne, Antibacterial, *Bacillus aerius*, *Sinularia* sp., skin desease

Introduction

Acne problems are experienced by more than 80% of the population aged 12-44 years. Generally, acne occurs during puberty (8-9 years) when the production of androgen hormones increases dramatically and results in increased secretion of sebum keratin (Winarno et al., 2014). Acne (acne vulgaris) is a human skin disease in the form of inflammation caused by oil accumulation in the polysebaceous glands, causing the growth of acne-causing bacteria (Karimah and Aryani, 2021), characterized by the presence of comedones, papules, pustules, nodules, cysts and scars (Saragih, Opod, and Pali, 2016). Bacteria that cause acne consist of *Staphylococcus epidermidis* (Severn and Horswill, 2022), *Staphylococcus aureus* (Hamida et al., 2022), and *Propionibacterium acnes* (Motosko et al., 2019). Acne treatment methods can be done by suppressing the growth of acne-causing bacteria and reducing inflammation in acne. The biodiversity that exists in Indonesia, especially in the oceans, can certainly be explored to obtain bioactive compounds that can be used for the treatment of acne.

Indonesia is a maritime country with a very abundant diversity of marine life. One of the many types of marine life is coral reefs. Coral reefs are marine ecosystems where marine biota live with ecological, economic, tourist, chemical and biological functions (Salanggon & Finarti, 2016). One of the biotabiotas that inhabit coral reefs is soft coral. Tanod et al. (2015) stated that soft corals (phylum Cnidaria) are invertebrate animals that live in coral reef ecosystems. Soft corals (Octocorallia, Alcyonacea) are included in the Coelenterata animals that live in tropical and subtropical shallow waters (Manuputty, 2016). According to statistical data from the Karimunjawa National Park Agency for 2019, there are 23 species of soft corals whose potential has not been explored in biotechnology.

Soft corals produce bioactive compounds as a means of self-protection (Tanod et al., 2019a). Bioactive compounds produced by soft corals can act as antibacterials in the form of steroids, terpenoids, and steroid glycosides (Wang et al., 2012). *Sinularia* sp. produce bioactive compounds with antibacterial properties (Salanggon et al., 2020; Manuputty, 2016). *Sinularia* sp. also has antiviral activity (Elkhouly et al., 2022), anti-inflammatory and anticancer (Chen et al., 2022), NFkB and iNOS inhibitors (Riyadi et al., 2019) and NO inhibitors (Fattorusso et al., 2011 and Putra et al. al., 2012). To facilitate the multiplication of bioactive compounds from *Sinularia* sp. This can be done with the help of the symbiont bacteria from this soft coral. Symbion bacteria are organisms that attach and interact with the host without causing negative impacts. The term bacterial symbiont was originally introduced by Frank, Van Beneden, and De Bary in 1870 (Prigot-Maurice et al., 2022). Mutualistic symbiotic relationship between *Sinularia* sp. with its symbiontic bacteria, *Sinularia* sp. provide a place to live and protect the symbiontic

Commented [V11]: In marine host-microbial interaction, you can use "symbiont" terminology if you already confirm that your bacteria has symbiosis relationship with its host. If you did not do any confirmation test, please use "associate" as your terminology. You can change become ***Sinularia–associated bacteria***. Especially, you got *Bacillus.* After you decide the terminology you want to use, please check your whole manuscript then change them.

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Commented [V113]: Please check again the meaning of symbiont. You must to consider that there is parasitism symbiosis. This symbiosis gives negative impacts to the host.

bacteria, while the symbio~~n~~tic bacteria help the process of nutrient cycling and as a source of secondary metabolites (Hillman & Goodrich-Blair, 2016). The bioactive compounds produced by the symbio~~n~~tic bacteria are similar of ~~the~~ same as their host (Paul et al., 2021). Symbion bacteria have a short life cycle so that the production of bioactive compounds can be done quickly without requiring large areas of land. The bioactive compounds produced by *Sinularia* sp. in the form of potential as anti-inflammatory and antibacterial, allowing this soft coral to be used in the treatment of acne. Symbion bacteria from *Sinularia* sp. can produce the same bioactive compounds as those produced by the soft corals and will assist in the production of bioactive compounds. Therefore, this study will explore the potential of the soft coral symbiont bacteria *Sinularia* sp. as from ~~karimunjawa~~ Karimunjawa as an antibacterial against *P. acnes* and *S. epidermidis*. This study will also carry out qualitative tests of bioactive compounds (flavonoids, alkaloids, steroids, terpenoids, phenolics) and molecular identification of isolates that have the potential to have antibacterial activity.

Methods

Sampling

Sampling was carried out in Karimunjawa, Jepara, Central Java with a purposive sampling method, namely determining the location by observing the presence of soft corals by means of scuba diving. Soft coral samples that have been taken, stored in a container filled with sterile sea water.

Soft Coral Identification

Identification of soft coral species was carried out with the help of Soft Coral and Sea Fans books supported by statistical data from the Karimunjawa National Park Office for 2019.

Isolation of Soft Coral Symbiont Bacteria

Isolation of symbio~~n~~tic bacteria begins by taking 3 grams of soft coral polyps. The sample surface was cleaned using sterile sea water. The soft corals were crushed with sterile mortar. Soft corals that have been mashed are taken as much as 1 gram and dissolved in 9 ml of distilled water for a 10^{-1} dilution. Next, 1 ml was taken from the first tube and put in 9 ml of sterile distilled water for a 10^{-2} dilution. Sample dilution was carried out up to 10^{-8} dilution. At dilutions 10^{-6}, 10^{-7}, 10^{-8}, 1 ml was taken using a micropipette and inoculated into a petri dish with marine agar media using the spread plate method (Setyati et al., 2016). The purification of the symbiont bacteria was carried out using the streak plate method with the help of round loops~~.~~ (Widyaningsih and Sa'adah, 2018). The pure bacterial culture was transferred to the agar slant~~ing~~ media for marine agar in a test tube as culture stock and working culture. Culture maintenance ~~is~~ was carried out by rejuvenating isolates every four weeks (Harpeni, 2007).

Characterization of Soft Coral Symbiont Bacterial Isolate

Observation of macroscopic morphology was carried out by observing bacterial colonies. Colony observations included colony shape, colony color, colony surface, margins, and elevation.

Microscopic morphological observation~~s~~ ~~were~~ was carried out using the Gram stain method. The purpose of Gram staining is to see the shape of the bacterial cell and to distinguish between Gram positive and Gram negative bacteria. The Gram stain procedure begins with sterilizing the slide using 70% alcohol. The glass object was given drops of distilled water and one ose of bacterial isolates, then mixed and fixed on a spirit lamp. Crystal violet dye (Gram A) was dropped on the fixation result and left for 1 minute then rinsed with distilled water. Lugol's solution (Gram B) was dripped onto the preparation, left for 1 minute and then rinsed with distilled water. 96% alcohol (Gram C) was dripped onto the preparation, left for 30 seconds and then rinsed with distilled water. Safranin (Gram D) was dripped onto the preparation, left for 1 minute and then rinsed with distilled water. Stained preparations were observed under a microscope and could be clarified with the addition of immersion oil (Wantania et al., 2016).

Antibacterial Activity Test with Kirby Baueur Method

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Commented [V116]: Please write the example of compounds that have been reported from the host and its associated/symbiotic bacteria.

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Commented [V118]: Please provide maps or GPS coordinate. After I checked, Karimunjawa is part of National Park. Please provide the number of sampling permit.

Commented [V119]: Please provide the picture of your sample underwater since you did SCUBA diving for sampling.

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The antibacterial test was carried out using the Kirby Baueur method~~, namely the agar diffusion method~~ with paper discs. The test was carried out by growing the test bacteria on MHA media. The test bacterial suspension was inoculated by means of a swab on MHA media. Paper discs (6mm) were dripped with 20µL of a suspension of soft coral symbiont bacteria, chloramphenicol (concentration?), and sterile distilled water. Chloramphenicol as a positive control and sterile distilled water as a negative control. Paper discs that have been dripped ~~are~~ were placed on MHA media which ~~has~~ have been overgrown with test bacteria (Dewi et al., 2020). Then incubated for 48 hours at 28oC. Antibacterial activity was observed by looking at the clear zone or inhibition zone that was formed (Kartika, 2017).

Analysis of Antibacterial Compounds

Analysis of bioactive compounds using the Gas Chromatography Mass Spectrometer (GC-MS) method. The tools used are GC/MS Thermo Scientific with Trace ISQ7000 MS spec and Trace 1310 GC. The column used was Agilent 190915-433UI: 0236716H HP-5MS UI with a size of 30 m x 250 µm x 0.25 µm. The carrier gas used is helium gas. The temperature used is 100°C with an average increase of 10°C/minute and the maximum temperature is 300°C. Column flow rate 1 ml/min. **How did you determine the compounds? Why you did not explain it here?**

Molecular Identification

Bacterial isolates with the best antibacterial activity and producing bioactive compounds were identified molecularly. Molecular identification using 16S rRNA gene markers was carried out based on the method of Noer (2021) and Nafia (2021) with modifications. DNA extraction was carried out using the InstaGeneTM Matrix kit protocol. DNA amplification using the polymerase chain reaction (PCR) ~~method with the help of a thermocycler~~Thermocycler. Amplification of the bacterial genomic DNA against the 16S rRNA gene was performed using a forward primer ~~27f~~ 27F (5'- AGA GTT TGA TCA CTG GCT CAG - 3') and a reverse primer ~~1492r~~ 1492R (5'- TAC GGC TTA CCT TGT TAC GA -3'). PCR master mix was made with a total volume of 50 µL consisting of 2 µL forward primer, 2 µL reverse primer, 2 µL DNA template, 25 µL My Taq HS red DNA polymerase (bioline), and 19 µL ddH2O. The mixture is homogenized and spindown is then put in a thermocycler (Pahriyani, 2020). PCR products were confirmed using electrophoresis and visualized with UVIDoc HD5.

DNA sequencing by 1st Base through PT. Indonesian Science Genetics. The DNA sequencing process was carried out using the dideoxy sanger method. The DNA sequences obtained were processed using the Basic Local Alignment Search Tool (BLAST) database tracking program on the website of the National Center for Biotechnology Information, National Institute for Health, USA, namely www.ncbi.nlm.nih.gov. Sequences were processed with the Mega11 program. Sequence characterization was performed by aligning

Results and Discussion

Characteristics of *Sinularia* sp. ~~Soft Coral~~ Symbion Bacteria.

Isolation of symbiont bacteria from soft coral *Sinularia* sp. carried out by serial dilution from 10^{-1} to 10^{-8} using sterile seawater as a solvent. Nine bacterial symbionts *Sinularia* sp. were successfully isolated with different colony and cell morphology. Each symbiont bacterial isolates ~~is~~ was named with the code LA1, LA2, LA3, LA5, LA6, LA7, LA8, LA10, and LA11. The symbiont bacterial isolates were observed macroscopically (Figure 1) and microscopically (Figure 2). Following are the results of the characterization of the soft coral symbiont bacteria *Sinularia* sp. (Table 1).

Table 1. Characteristic data of soft coral symbiont bacteria *Sinularia* sp.

Isolate	Shape	Margin	Elevation	Texture	Colony Color	Gram	Cell Shape
LA1	irregular	lobate	umbonate	dry	light yellow	+	rod

Commented [V124]: What is the origin of your pathogens? Is it a type strain? Clinical strain? How did you get these strains? Then, is it antibiotic sensitive or drug resistant? Please give more information.

Commented [V125]: Why? If you want to use a negative control, you should use the culture media, not the sterile water. Because you need to confirm whether the antibacterial substance was produced by the bacteria or from the media.

Commented [V126]: Are you sure? So the pathogens have been grown on the agar then you add your symbiotic bacterial suspension, then you incubated them again for 48h? So, the pathogens grew more than 48h on the agar? Are you sure this method from Kirby? Please read this: https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf

Commented [V127]: Why suddenly GC-MS analysis appear? What did you analysis? The living cells? Or did you cultivate your strain to produce crude extract then the crude extract was analysed using GC-MS? Please clarify this step.

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Commented [V131]: What do you mean use sterile seawater as solvent? Solvent is a terminology to refer certain liquid or gas that use for metabolite extraction/fractionation.

Commented [V132]: Please consider to write: bacterial strains rather than bacterial isolates.

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LA2	round	irregular	umbonate	mucoid	milk white	+	rod
LA3	irregular	lobate	flat	moist	beige	-	spherical
LA5	irregular	irregular	convex	moist	beige	+	spherical
LA6	irregular	lobate	flat, raised margin	mucoid	yellow	+	rod
LA7	irregular	lobate	umbonate	dry	yellow	+	rod
LA8	irregular	undulate	lobate	dry	beige	-	rod
LA10	round	smooth	convex	moist	milk white	-	rod
LA11	round	irregular	raised	mucoid	white	+	rod

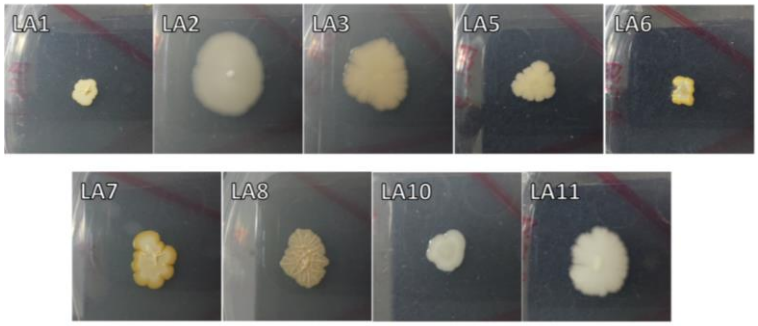

Figure 1. Results of macroscopic observations of *Sinularia* sp. soft coral symbiont bacterial.

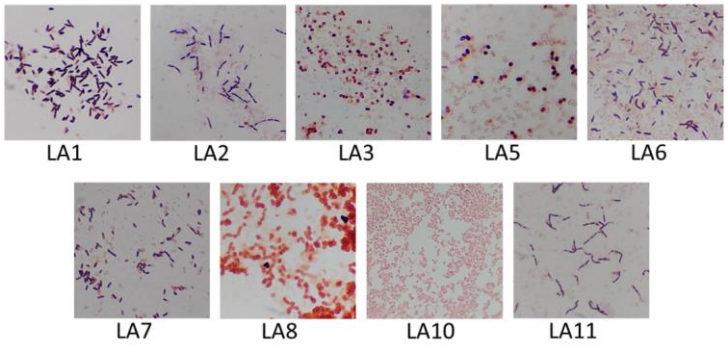

Figure 2. The results of Gram staining of *Sinularia* sp. soft coral symbiont bacterial.

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Macroscopic observation~~s~~ by looking at the morphology of the symbiont bacterial colonies and based on the Microbiology Laboratory Theory and Application Leboffe and Pierce guidebook (2012). Microscopic observation of the symbiont bacteria was carried out by looking at the cell morphology and the grouping of Gram-positive or Gram-negative bacteria. Appearance of Gram stain, Gram negative bacterial cells are red and Gram positive bacterial cells are purple. There are differences in the peptidoglycan wall between Gram positive and negative bacteria. According to Tripathi and Sapra (2020), Gram staining is a method used to distinguish between Gram positive and Gram negative bacteria. Gram positive bacteria will appear purple and Gram negative bacteria will appear red when observed under a microscope. The initial dye for Gram staining is crystal violet. Iodine material will

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form a violet-iodine crystal complex to prevent the purple color from fading easily. The decolorizer process with the addition of ethanol and acetone was carried out to remove the color from the crystal violet. The principle of Gram staining is based on the ability of the bacterial cell wall to maintain the crystal violet color during treatment. The cell wall of Gram positive bacteria has a higher peptidoglycan content than Gram negative bacteria which has a high lipid content. Gram positive and Gram negative bacteria are able to absorb crystal violet color. The lipid layer on the cell wall of Gram negative bacteria is easily shed so that the crystal violet color will be shed during the decolorization process. The addition of safranin dye to the sample aims to give a red color to Gram negative bacteria.

The results of observations of the symbiont bacteria *Sinularia* sp. which is summarized in the table shows that there are 3 isolates with round shapes and 6 isolates with irregular shapes. Colony margins varied greatly, 4 isolates had lobate margins, 3 isolates had irregular margins, 1 isolate had undulate margins, and 1 isolate had smooth margins. There are also isolates with dry, mucoid, and moist textures. Observations from Gram staining of the symbiont bacteria *Sinularia* sp. showed that there were 3 Gram negative bacteria and 6 Gram positive bacteria. The diversity of bacteria obtained ~~will be~~were continued for antibacterial activity tests against *P. acnes* and *S. epidermidis*.

Antibacterial Activity Test

Antibacterials are compounds that can inhibit the growth or kill bacteria. In this study, the antibacterial activity test of the symbiont bacteria *Sinularia* sp. against acne-causing bacteria, namely ~~*Propionibacterium* *P. acne*~~ and ~~*Staphylococcus* *S. epidermidis*~~. P. acnes is a Gram-positive bacterium with bacilli-shaped cells. S. epidermidis is a Gram-positive bacterium with a coccal cell shape. The results of Gram staining for *P. acnes* can be seen in Figure 3 and *S. epidermidis* can be seen in Figure 4. Gram staining of the tested bacterial isolates aims to determine the purity of the test bacteria by looking at the uniformity of the test bacterial cells.

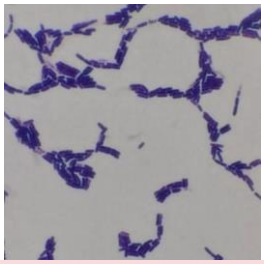

Figure 3. *Propionibacterium acnes* cell morphology

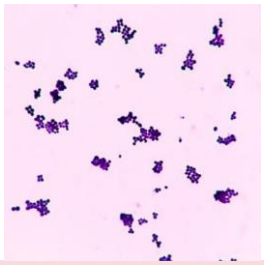

Figure 4. *Staphylococcus epidermidis* cell morphology

For the initial stage, an antibacterial screening process will be carried out to obtain isolates of the symbiont bacteria *Sinularia* sp. which can produce antibacterial compounds. The method used is disk

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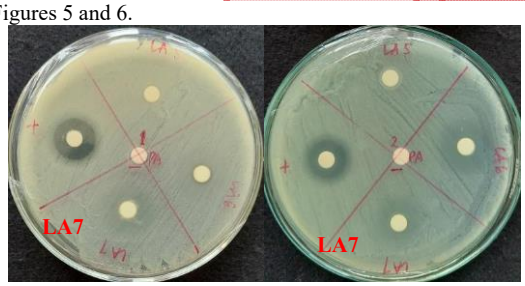
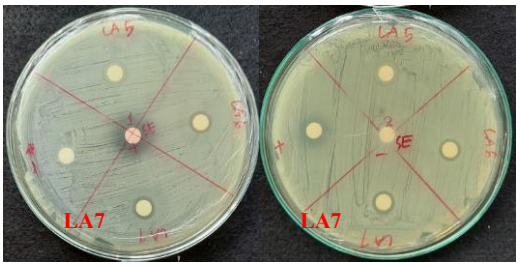
diffusion. Bacteria that have antibacterial activity will inhibit the growth of pathogenic bacteria, as evidenced by the clear zone that forms around the disc paper. The results of the antibacterial screening ~~of the antibacterial activity~~ of the symbiont bacteria *Sinularia* sp. against *P. acnes* and *S. epidermidis* ~~can be seen~~is shown by ~~in~~ Figures 5 and 6.



(a) (b)

Figure 5. The results of the screening test for antibacterial activity of the *Sinularia* sp. symbiont bacteria against *S. epidermidis* after X h of incubation (a) replicate 1 (b) replicate 2



(a) (b)

Figure 6. The results of the screening test for antibacterial activity of the *Sinularia* sp. symbiont bacteria against *P. acnes* after X h of incubation (a) replicate 1 (b) replicate 2

The results of the screening of the antibacterial activity of the symbiont bacteria *Sinularia* sp. against *P. acnes* and *S. epidermidis* showed that there were several isolates that formed inhibition zones. The inhibition zone resulting from the antibacterial test results indicates the activity of inhibiting the growth of the tested bacteria. Isolate with the best inhibition zone was re-tested for its antibacterial activity to categorize the level of its antibacterial activity strength. Data from the screening results for antibacterial activity are summarized in ~~table~~ Table 2.

Table 2 Antibacterial activity screening results

Isolate Code	Inhibition Zone	
	Propionibacterium acnes	*Staphylococcus epidermidis*
LA1	+	+
LA2	+	-
LA3	-	-
LA5	-	+
LA6	+	+
LA7	+	+
LA8	-	-

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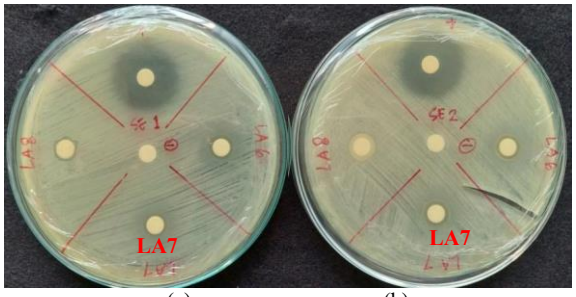
LA10	-	-
LA11	-	-

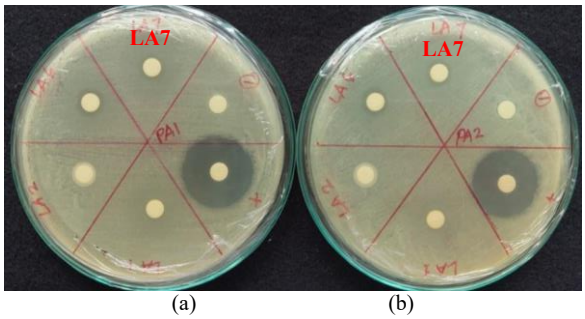
Note:
(+) inhibition zone formed (positive for antibacterial activity)
(-) no inhibition zone is formed (negative has antibacterial activity)

Based on the data in table 2, it can be seen that the isolates LA1, LA2, LA6, and LA7 have the potential to inhibit the growth of *P. acnes* because they form a clear zone around the paper disk. It can also be seen that the isolates LA6, LA7, and LA11 were able to inhibit the growth of S. epidermidis because they formed an inhibition zone around the paper disk. Isolates that have the potential for antibacterial activity will be tested for antibacterial activity to determine the strength of the antibacterial activity so that the level of inhibition can be categorized based on the size of the inhibition zone formed. The inhibition zone formed around the disc paper was measured with a vernier caliper. The results of the antibacterial activity test can be seen in Figures 7 and Figures 8. The results of measuring the diameter of the inhibition zone are summarized in tables 3 and 4.



(a) (b)

Figure 7. Antibacterial activity test results of isolates LA1, LA3, LA5, LA6, LA7, LA8, and LA11 against *S. epidermidis* (a) Replication 1 (b) Replication 2



(a) (b)

Figure 8. Results of antibacterial activity test of isolates LA1, LA2, LA6, and LA7 against *P. acnes* (a) Replication 1 (b) Replication 2

Table 3. The results of the antibacterial activity test of the symbiont *Sinularia* sp. Isolate code LA1, LA3, LA5, LA6, LA7, LA8, and LA11 against *S. epidermidis*.

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Isolate Code	Inhibition Zone Diameter (mm)		Average (mm) please add the standard deviation (±)
	Replication 1	Replication 2	
LA1	13,2	15,6	14,4
LA5	6,3	4,6	5,5
LA11	5,1	4,5	4,8
LA6	13,9	14,3	14,1
LA7	14,7	14,4	14,6
Positive control	19,6	17,9	18,75
Negative control	0	0	0

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Table 4. The results of the screening of the antibacterial activity of the symbiont bacteria *Sinularia* sp. code isolates LA1, LA2, LA6, and LA7 against *P. acnes*.

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Isolate Code	Inhibition Zone Diameter (mm)		Average (mm) please add the standard deviation (±)
	Replication 1	Replication 2	
LA1	13,6	15,2	14,4
LA2	5,5	5,7	5,6
LA6	15,4	15,5	15,5
LA7	17,6	15,7	16,6
Positive control	19,3	17,7	18,5
Negative control	0	0	0

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Based on the data from the antibacterial activity test results in Tables 3 and 4, it can be seen that several isolates formed ~~an~~ inhibition zone. The largest diameter inhibition zone was formed in LA7 isolate. The size of the inhibition zone formed is influenced by several factors, namely competition between the symbiont bacteria *Sinularia* sp. and pathogenic bacteria as well as the production of secondary metabolites in the form of bioactive compounds. According to Chevrette et. al. (2022), secondary metabolites produced by bacteria are the main source of antibiotics and other bioactive compounds. Within the microbial community, these molecules can mediate interspecies interactions and responses to environmental changes.

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Based on the antibacterial activity test against *S. epidermidis*, LA7 isolate formed an inhibition zone of 14.6 mm which indicates that the bacteria has moderate category of antibacterial activity. In P. acnes, LA7 isolate formed an inhibition zone of 16.6 mm indicating that LA7 isolate has strong antibacterial activity. According to Cita et. al. (2017) antibacterial activity based on the size of the inhibition zone formed around the disc paper was classified as strong for inhibition zone diameter (id) > 16.0 mm, medium (good) for diameters ranging from 11 to 16 mm, weak for diameters 7–11 mm and no activity for diameters <7 mm. From the results of the antibacterial activity test, LA7 isolate with the best activity will continue to identify species based on the 16S rRNA gene. LA7 isolate is a Gram positive bacterium with bacilli form.

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Analysis of Bioactive Compounds with GC-MS method

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Gas chromatography and mass spectrometry have broad analytical applications that can be used as summaries and mixtures of several components of chemical compounds (Chirumamilla et al., 2022). The results of the GC-MS crude extract of isolate LA7 are displayed in the form of a chromatogram (Figure 9). After processing the data, there were 81 compounds detected from Isolate LA7 (Table 5).

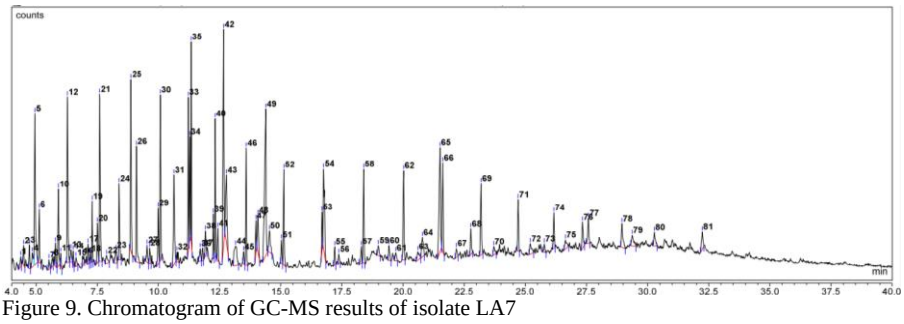

Figure 9. Chromatogram of GC-MS results of isolate LA7

Table 5. Compound from GC-MS analysis isolate LA7

Peak	Ret.Time (min)	Compound	Chemical Formula	Rel. Area (%)
1	4,37	Octadecane, 6-methyl-	$C_{19}H_{40}$	0,32
2	4,48	1-Octadecanesulphonyl chloride	C18H37ClO2S	0,39
3	4,72	2-Tridecen-1-ol, (E)-	C13H26O	0,46
4	4,84	3-Trifluoroacetoxydodecane	C14H25F3O2	0,52
5	4,95	Dodecane	C12H26	3,94
6	5,13	Undecane, 2,6-dimethyl-	C13H28	1,01
7	5,52	Undec-10-ynoic acid, dodecyl ester	C23H42O2	0,25
8	5,67	2-Piperidinone, N-[4-bromo-n-butyl]-	C9H16BrNO	0,27
9	5,79	Undecane, 4,8-dimethyl-	C13H28	0,45
10	5,91	Octane, 2,3,7-trimethyl-	C11H24	1,52
11	6,01	Tetradecane, 2,6,10-trimethyl-	C17H36	0,24
12	6,28	Tridecane	C13H28	3,54
13	6,43	Bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	C11H10	0,41
14	6,52	Octadecane, 6-methyl-	C19H40	0,20
15	6,63	Tetradecane, 2,6,10-trimethyl-	C17H36	0,26
16	6,90	9,12,15-Octadecatrienoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, (Z,Z,Z)-	C25H40O6	0,32
17	7,12	Tetradecane, 2,6,10-trimethyl-	C17H36	0,42
18	7,21	Octadecane, 6-methyl-	C19H40	0,19
19	7,29	Dodecane, 2,6,10-trimethyl-	C15H32	0,94
20	7,50	4-Trifluoroacetoxytetradecane	C16H29F3O2	0,93
21	7,60	Tetradecane	C14H30	3,62
22	7,88	2,6,10-trimethylundecanoic Acid, 2,2,2- trifluoroethyl ester	C16H29F3O2	0,39
23	8,27	2-Trifluoroacetoxypentadecane	C17H31F3O2	0,46
24	8,39	Heptadecane, 2,6,10,14-tetramethyl-	C21H44	1,56
25	8,87	Pentadecane	C15H32	3,30
26	9,10	Butylated Hydroxytoluene	C15H24O	2,17

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27	9,54	1-Hexadecanol, 2-methyl-	C17H36O	0,45
28	9,64	Tetradecane, 2,6,10-trimethyl-	C17H36	0,42
29	10,00	1-Decanol, 2-hexyl-	C16H34O	1,20
30	10,08	Hexadecane	C16H34	2,88
31	10,64	Tetradecane, 2,6,10-trimethyl-	C17H36	2,75
32	10,76	1-Hexadecanol, 2-methyl-	C17H36O	0,37
33	11,22	Heptadecane	C17H36	2,35
34	11,29	Pentadecane, 2,6,10-trimethyl-	C18H38	1,60
35	11,34	Dodecane, 1-cyclopentyl-4-(3-cyclopentylpropyl)-	C25H48	2,82
36	11,70	1-Hexadecanol, 2-methyl-	C17H36O	0,43
37	11,81	Octadecane, 6-methyl-	C19H40	0,31
38	11,92	Ethanol, 2-(octadecyloxy)-	C20H42O2	0,65
39	12,24	5-Octadecene, (E)-	C18H36	0,74
40	12,32	Eicosane	C20H42	2,21
41	12,43	Tetradecane, 2,6,10-trimethyl-	C17H36	0,69
42	12,66	Bicyclo[3.3.1]nonane-2,8-dione	C9H12O2	4,93
43	12,78	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C11H18N2O2	2,61
44	13,15	1,3,2-Dioxaborinane, 2,4-diethyl-5-methyl-6-propyl-	C11H23BO2	1,53
45	13,49	Tetradecane, 2,6,10-trimethyl-	C17H36	0,33
46	13,59	Eicosane	C20H42	2,31
47	13,98	Hexadecanoic acid, methyl ester	C17H34O2	0,63
48	14,07	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C11H18N2O2	1,23
49	14,39	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C11H18N2O2	6,15
50	14,54	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C11H18N2O2	0,89
51	15,03	1-Decanol, 2-hexyl-	C16H34O	0,52
52	15,13	Eicosane	C20H42	2,08
53	16,69	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	0,82
54	16,76	Eicosane	C20H42	3,07
55	17,21	Heptadecanoic acid, 16-methyl-, methyl ester	C19H38O2	0,43
56	17,38	Tetradecane, 2,6,10-trimethyl-	C17H36	0,26
57	18,30	1-Decanol, 2-hexyl-	C16H34O	0,44
58	18,39	Eicosane	C20H42	2,08
59	18,99	Tetradecane, 2,6,10-trimethyl-	C17H36	0,34
60	19,42	7-Methyl-Z-tetradecen-1-ol acetate	C17H32O2	0,43
61	19,70	tert-Hexadecanethiol	C16H34S	0,33
62	20,03	Eicosane	C20H42	2,22
63	20,61	tert-Hexadecanethiol	C16H34S	0,46
64	20,79	Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'a,10a)-	C33H37N5O5	0,93
65	21,51	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	C14H16N2O2	4,03
66	21,63	Eicosane	C20H42	2,01

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67	22,18	tert-Hexadecanethiol	C16H34S	0,29
68	22,77	Octadecanoic acid, 2-propenyl ester	C21H40O2	0,70
69	23,19	Eicosane	C20H42	1,84
70	23,71	tert-Hexadecanethiol	C16H34S	0,33
71	24,71	Eicosane	C20H42	1,24
72	25,21	tert-Hexadecanethiol	C16H34S	0,55
73	25,77	Tetrapentacontane, 1,54-dibromo-	C54H108Br2	0,27
74	26,17	Tetradecane, 2,6,10-trimethyl-	C17H36	0,90
75	26,65	tert-Hexadecanethiol	C16H34S	0,49
76	27,34	9-Octadecenamide	C18H35NO	0,87
77	27,58	Tetradecane, 2,6,10-trimethyl-	C17H36	0,75
78	28,95	tert-Hexadecanethiol	C16H34S	0,96
79	29,37	tert-Hexadecanethiol	C16H34S	0,61
80	30,27	tert-Hexadecanethiol	C16H34S	0,53
81	32,24	7-Methyl-Z-tetradecen-1-ol acetate	C17H32O2	0,66

Based on the GC-MS data, there were identified antibacterial compounds at high concentrations namely Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- (6.15%). Several studies related to the compound Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- have been carried out, it was found that the compound has antibacterial and antioxidant activity (Makuwa & Serepa-Dlamini, 2021; Kiran et al., 2018). The second bioactive compound was Bicyclo[3.3.1]nonane-2,8-dione (4,93%) which potential as anticancer chemotherapeutics (Roy et al., 2023). The third was Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-(4,03%) which has strong antioxidant activity (Gopi et al., 2014; Balakrishnan et al., 2015). Based on this data, it proved that the isolate LA7 has potential against acne causing bacteria (*P. acnes* and *S. epidermidis*).

Molecular 16S rRNA Identification

Bacteria with isolate code LA7 which has the best antibacterial activity against *P. acnes* and *S. epidermidis* were identified molecularly. Species identification using 16S rRNA gene markers. To obtain DNA isolate LA7, DNA extraction was performed using the InstaGenTM matrix protocol. DNA purity was measured by a spectrophotometer nanodrop. The results of measuring the purity of LA7 isolate can be seen in table 6.

Table 6. Results of nanodrop spectrophotometry of DNA isolate LA7

Sample	Concentration (ng/µl)	Wavelength		Ratio
		$\lambda 260$	$\lambda 280$	$A\lambda 260/A\lambda 80$
LA7	342,3	6,846	3,252	2,11

The table shows that the purity of the DNA isolate LA7 is 2,11. Extracted DNA can be used as a template in PCR 16S rRNA gene amplification. According to Hindash and Hindash (2022) DNA purity can be determined quantitatively with a nanodrop spectrophotometer at an OD ratio of 260/280. Based on the OD 260/280 ratio, DNA is declared pure from protein if it is less than 1.8 and pure from RNA if it is more than 2.0. **So, what does the meaning of your 2.11 value? Is it pure from protein and RNA contamination? Please write it clearly.**

The results of DNA isolation from LA7 isolate were amplified by the 16S rRNA gene by PCR. To determine the success of amplification of LA7 isolate, agarose gel electrophoresis was performed. According to Balakrishnan et. al. (2022), the amplicon size of the 16S rRNA gene is in the range of 1500 bp which consists of a conserved region. The amplification of the 16S rRNA gene was declared successful if the amplicon visualized by gel electrophoresis showed a size of 1500 bp. Visualization of

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the electrophoretic gel in Figure 10 shows the amplicon size of isolate LA7, which is 1500 bp, indicating that the 16S rRNA gene was successfully amplified. The results of the amplification of LA7 isolate will then be sequenced to determine the nucleotide base sequence. The results of the 16S rRNA gene amplification can be seen in Figure 10.

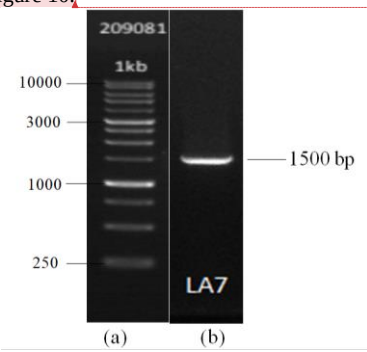

Figure 10. Electrophoresis of 16S rRNA gene amplification using PCR. (a) DNA Ladder 1kb (b) Amplicon band 16S rRNA Isolate LA7

The sequencing results of LA7 isolates were analyzed using BLAST (Basic Local Alignment Search Tools) on the NCBI online page. The BLAST analysis aims to compare the LA7 sequence with the DNA sequence data in GenBank. The results of the BLAST analysis are listed in ~~table~~ Table 7.

Table 7. Results of BLAST analysis of bacterial isolate LA7

~~Name of Bacteria~~Closest species	*Max score*	*Total Score*	*Query Cover*	E-Value	*Per. Ident*
Bacillus aerius	2100	2100	100%	0.0	98.17%
Bacillus stratosphericus	2097	2097	100%	0.0	98.08%
Bacillus altitudinis	2095	2095	100%	0.0	98.08%
Bacillus pumilus	2095	2095	100%	0.0	98.08%
Bacillus aerophilus	2095	2095	100%	0.0	98.08%

The results of the BLAST sequence of isolate LA7 showed the highest homology value, namely 98.17%, which means that LA7 has species-level similarities with *Bacillus aerius*. Moritania et al. (2019) that in the BLAST results, homology values (percent identity) of more than 97% can represent similarities at the species level. The homology (percent identity) value of 93% - 97% can represent identity at the genus level but differs at the species level. Percent identity with a value below 93% means the possibility of a new species whose nitrogen base sequence has not been included in the Genbank database. Based on ~~table~~ Table 7, isolate LA7 has the highest similarity with *Bacillus aerius* with a maximum score of 2100, a total score of 2100, 100% query coverage, 0.0 E-value, and 98.17% identity percentage. According to Claverie and Notredame (2003), the maximum score and total score are the number of alignment segments from the database sequence that match the nucleotide sequences. The score value indicates the accuracy of the alignment of the unknown nucleotide sequence with the data in GenBank, the higher the value the higher the homology level of the two sequences. The E-value is an estimated value that provides a statistically significant measure of both sequences. A higher E-value indicates a lower level of homology between sequences, while a low E-value indicates a higher level of homology between sequences. The E-value is 0 (zero) indicating that the query sequence is similar to the sequence in GenBank. Query coverage shows the percentage of database covered by queries.

After the LA7 sequences were analyzed with BLAST, phylogenetic tree analysis was performed. Phylogenetic tree analysis using the "Neighbor Joining" approach, and boostrap 1000X. According to

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Rusinko and McPartlon (2017), ~~neighbor~~ Neighbor ~~joining~~ Joining (NJ) is a taxonomic method that uses distance as a principle for grouping taxa. The grouping of these taxa is based on calculating the evolutionary distance of pairs of operational taxonomic units where each branch of the phylogenetic tree evolves at a different speed. According to Dey et. al. (2022), bootstrap values are used to estimate the confidence level of a phylogenetic tree. The higher the bootstrap value, the higher the level of confidence in the reconstructed tree topology. This is due to random effects in the distribution of characters in the data. The phylogenetic tree of isolate LA7 can be seen in Figure 11.

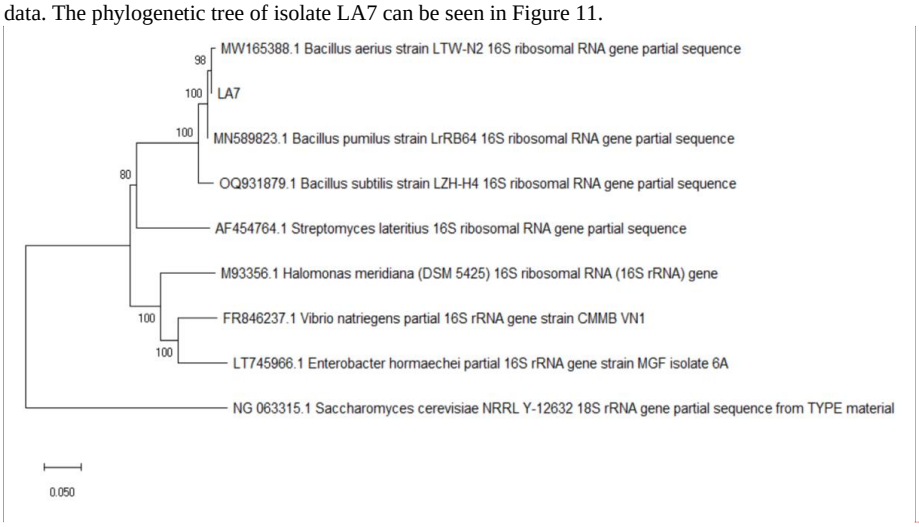

Figure 11. The phylogenetic tree of isolate LA7 showed it has high similarity with *B. aerius*. The evolutionary history was inferred using the Neighbor-Joining method (Saitou N. and Nei M., 1987). The optimal tree with the sum of branch length = 1.54531298 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004). and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There were a total of 1760 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

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The phylogenetic analysis of LA7 isolates was carried out by comparing the bacterium with several other species, consisting of in group and out group. The in group was selected from the BLAST results, namely *Bacillus aerius* and *Bacillus pumilus*. In the group of the genus Bacillus used is *Bacillus subtilis*. The other in group used was bacteria that had in common with isolate LA7, antibacterial activity against *P. acnes* and *S. epidermidis*. These bacteria are *Streptomyces lateritius*, *Halomonas meridian*, *Enterobacter hormaechei*, and *Vibrio natriengens*. The selection of the out group came from a fungus, namely *Saccharomyces cerevisiae*. Subari et al. (2021) stated that the purpose of using outgroups in constructing phylogenetic trees is to identify primitive (plesiomorphic) and derived (apomorphic) characteristics of ingroup groups, as well as to establish a starting point in constructing a phylogenetic tree.

Based on the cladogram in Figure 11, isolate LA7 has a close relationship with *Bacillus aerius* with a bootstrap value of 98. This bootstrap value has a high level of confidence. According to Subari et al. (2021) the bootstrap value is the value used to test how well the model data set is used, if the bootstrap value is low then the sequence of the analysis to obtain a phylogenetic tree cannot be trusted. A

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phylogenetic tree that has a high and good level of confidence is a phylogenetic tree with a bootstrap value above 70.

Bacillus aerius is a gram-positive ~~bacillus~~ bacteria in the form of bacilli. *Bacillus aerius* bacteria are halotolerant, able to live in places with a salt concentration of 0-30% (Chauduri et al., 2021). *Bacillus aerius* is known as a gastrointestinal microbe because it is found in the digestive tract of catfish (Meidong et al., 2018), sea sponges (Maharsiwi et al., 2020) and grouper fish (Soltani et al., 2019), the genus *Bacillus* is also widely known as a probiotic in fish. Gray (2023) found that *Bacillus aerius* can inhibit the replication of Methicillin-Resistant Staphylococcus aureus (MRSA). MRSA is known as a human skin pathogen which is very difficult to cure because of its resistance to the antibiotic methicillin. The study conducted by Galaviz-Silva et al. (2018) reported antimicrobial activity against *Staphylococcus aureus* by *Bacillus aerius* isolated from marine habitats in Mexico. The results of this study indicate that this marine bacterium has potential as an alternative in the development of new antimicrobials against other clinically important bacteria. According to Saun et al. (2014); Saun, Mehta and Gupta (2014), *Bacillus aerius* has not been reported to have directly antagonistic activity, but several reports revealed that *Bacillus aerius* produces a thermophilic lipase with antitumor properties, making it a possible candidate for therapeutic applications.

Based on several studies, *Bacillus aerius* has potential as a bacterium that can be developed in the medical field to fight pathogenic bacteria that cause disease in humans. The halotolerant nature of B. aerius facilitates the process of making pharmaceutical products under high salinity conditions.

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Commented [V167]: Please write your reference carefully. In Chauduri et al. I couldn't find any statement that refer to the ability of this bacterium to grow in salt concentration 0-30%. Moreover, in their table it is written that this bacterium tolerate with 5% NaCl.

Commented [V168]: But you also need to consider that this bacterium was firstly isolated from air. You can check this paper: https://www.microbiologyresearch.org/docserver/fulltext/ijsem/56/7/1465.pdf?expires=1698817414&id=id&accname=guest&checksum=3C325E56254973649072FF4B4011CDD5
It means, this bacterium is basically in the air and landed in any environment. Therefore, this bacterium might be not a symbiotic bacterium. Furthermore, in your reference from Gray (2023) stated that *B. aerius* is a human microbiota which isolated from skin and nostril.

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Conclusions

Soft coral symbiont bacteria *Sinularia* sp. with isolates code LA1, LA2, LA6, LA7 could inhibit the growth of *P. acnes* ~~and~~ while isolates LA1, LA3, LA5, LA6, LA7, LA11 could inhibit the growth of *S. epidermidis.* LA7 isolate had the best antibacterial activity with strong category of antibacterial activity against *P. acnes* and medium category of antibacterial activity against *S. epidermidis*. The results of species identification based on the 16S rRNA gene sequence showed that LA7 isolate was *Bacillus aerius* with a homology value of 98.17%. The result of GCMS, indicated that *Bacillus aerius* could produce Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl) (C11H18N2O2), Bicyclo[3.3.1]nonane-2,8-dione (C9H12O2), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3 (phenylmethyl)- (C14H16N2O2) which potential as anti-bacterial, anti-cancer and antioxidant activity.

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For the future work, it is important to do the Whole Genome Sequencing (WGS) of *B. aerius* to get the whole data of sequence and detect the potential gene cluster which encode those three potential compounds as anti-bacterial, anti-cancer and antioxidant activity. Furthermore, it is possible to do the cloning of the genes from three potential compounds and to determine the biosynthetic pathway. Then to apply the downstream processing of the product which could be used in the Industrial system

Commented [V169]: All compounds that you give in the Table 5 are common volatile organic compounds from any living organisms. Why do you want to clone them? Moreover the biosynthetic pathway for those compounds have been clearly identified.

Acknowledgments

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Title : **Antibacterial activity and molecular identification of soft coral *Sinularia* sp. symbiont bacteria from Karimunjawa Island against skin pathogens *Propionibacterium acnes* and *Staphylococcus epidermidis***

Section		Reviewer Suggestion
General Suggestion/Comment		You have a very interesting work. However, you need to rewrite your article using proper English. Some of terminologies also need to be confirmed. Some methodologies are need to be clarified.
1.	**Title**	• Please change the terminology of symbiotic bacteria since you did not conduct any test to confirm the symbiotic relationship between your strain with its host
2.	**Abstract**	• Check again the English
3.	**Introduction**	• Please read the comment. You need to strengthen your basic understanding about the concept of symbiosis and the ability to produce similar or same compounds. • Research objectives in the abstract and introduction part is little bit different
4.	**Material and Method**	• Please read the comment. • You need to clarify some of your protocols • Add information about the metabolite extraction
5.	**Result and Discussion**	• You need to rewrite some of them using proper English • You must curate your GC-MS data • You must make a new phylogenetic tree • Please read all my suggestions
6.	**Conclusion**	• Too long. Just fit it with your research objectives. Although you want to add some suggestions, you may add a new sub-chapter
7.	**References**	• Please write the reference according to the GDA of IOP
Reviewer Recommendation		Based on the above assessment, it was concluded that this manuscript: 1. Accepted with major revision
		Specific comment to chief editor (will not published to author):

Date: November 1th 2023
Reviewer. **MTS**