

Submission

The screenshot shows the submission dashboard for Biodiversitas Journal. The main content area is titled "Submission Files" and displays a table with the following data:

File Name	Date	Type
nikendharmayanti_20201008-Niken-Biodiversitas.docx	October 7, 2020	Article Text

Below the table, there is a "Download All Files" button. Underneath, the "Pre-Review Discussions" section is visible, showing a table of comments:

Name	From	Last Reply	Replies	Closed
Comments for the Editor	nikendharmayanti	2020-10-07 11:14 AM	0	<input type="checkbox"/>
Manuscript Submission	ayu	2020-10-09 05:26 AM	6	<input type="checkbox"/>

Perbaikan Editor

The screenshot shows the same submission dashboard as above, but with a "Manuscript Submission" modal window open. The modal contains the following information:

Participants
Assalamualaikum Niken - Dharmayanti, -est (nikendharmayanti)
Ayu Artuti (ayu)

Messages

Note	From
Dear author, Thank you very much for your manuscript submission. Unfortunately, your manuscript does not meet our requirements: - At least, to published in the Biodiversitas journal, you need to compose a minimum of 20 references which 80% of international scientific journals published in the last 10 years (2010-2020), and maximum 10% references in the local language (not English). And please write the references based on the author's guidelines. -This manuscript is too brief to be published in the Biodiversitas journal. At least, you need to compose a 2000 words article from the introduction to a conclusion (table and figure are excluded). Kindly check and correct accordingly Thank you	ayu 2020-10-09 05:26 AM
Assalamualaikum wr. wb. Dear Editor	nikendharmayanti 2020-10-12 04:19

Review 1

Journal Kelaut... x | gmail - Search x | [biodiv] Edito... x | Gmail - [biodi... x | whatsapp - 5... x | (6) WhatsApp x | NIKEN DHAR... x | Menu Admini... x | +

https://smujo.id/biodiv/authorDashboard/submission/6910

Biodiversity Journal of Biological Diversity Tasks 1 English View Site nikendharmayanti

6910 / Dharmayanti et al. / Antibacterial potential of symbiotic bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters Library

Workflow Publication

Submission Review Copyediting Production

Round 1 Round 2 Round 3

Round 1 Status
The submission must be resubmitted for another review round.

Notifications

[biodiv] Editor Decision	2020-11-09 04:03 PM
[biodiv] Editor Decision	2020-12-11 03:39 PM
[biodiv] Editor Decision	2020-12-30 09:52 AM
[biodiv] Editor Decision	2020-12-30 01:35 PM

Type here to search Daftar Pantau +2,56% 9:30 08/02/2023

Review 2

Journal Kelaut... x | gmail - Search x | [biodiv] Edito... x | Gmail - [biodi... x | whatsapp - 5... x | (6) WhatsApp x | NIKEN DHAR... x | Menu Admini... x | +

https://smujo.id/biodiv/authorDashboard/submission/6910

Biodiversity Journal of Biological Diversity Tasks 1 English View Site nikendharmayanti

6910 / Dharmayanti et al. / Antibacterial potential of symbiotic bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters Library

Workflow Publication

Submission Review Copyediting Production

Round 1 Round 2 Round 3

Round 2 Status
The submission must be resubmitted for another review round.

Notifications

[biodiv] Editor Decision	2020-11-09 04:03 PM
[biodiv] Editor Decision	2020-12-11 03:39 PM
[biodiv] Editor Decision	2020-12-30 09:52 AM
[biodiv] Editor Decision	2020-12-30 01:35 PM

https://smujo.id/biodiv/\$\$\$ca/\$\$\$/tab/author-dashboar.../author-dashboar-review-round-tab/fetch-review-round-info?submissionId=6910&stageId=3&reviewRoundId=2873

Type here to search Daftar Pantau +2,56% 9:30 08/02/2023

Copy Editing

The screenshot shows the OJS Copy Editing interface for submission 6910, titled "Antibacterial potential of symbiotic bacteria of brown algae (*Turbinaria concoides*) obtained from Indonesian waters". The interface includes a navigation menu on the left with "Submissions" and "Workflow" tabs. The main content area has tabs for "Submission", "Review", "Copyediting", and "Production". Under the "Copyediting" tab, there is a "Copyediting Discussions" section with an "Add discussion" button and a table with columns for Name, From, Last Reply, Replies, and Closed. Below this is a "Copyedited" section with a search button and a table listing the copyedited document: "34804-2 - editors_D220145-Turbinaria concoides - Dharmayanti - doc (2)", dated December 31, 2020, and categorized as "Article Text". The browser's address bar shows the URL: <https://smujo.id/biodiv/authorDashboard/submission/6910>. The taskbar at the bottom shows the system time as 9:31 on 08/02/2023.

Production

The screenshot shows the OJS Production interface for submission 6910. The "Status" is "Published". A prominent red banner across the top of the main content area reads: "This version has been published and can not be edited." The interface includes a navigation menu on the left with "Submissions" and "Workflow" tabs. The main content area has tabs for "Title & Abstract", "Contributors", "Metadata", "References", and "Galley". The "Title & Abstract" tab is active, showing the title "Antibacterial potential of symbiotic bacteria of brown algae (*Turbinaria concoides*) obtained from Indonesian waters" and the abstract text. The abstract text reads: "Abstract. Dharmayanti N, Anti A, Siregar RR, Spahutar Y, Permodi A, Siregar AM, Salampeppy RB, Sugulyanti, Nurbeni SZ, Purnamasari HB. 2021. Title: *Biodiversitas* 22: 373-378. Brown seaweeds have the potential to produce bioactive compounds. Bacteria associated with seaweeds are involved in the production of metabolites. Microbes may be present as a living symbiotic in association with other algae as epiphytes or endophytes. In this study, bacteria isolated from brown seaweed (*Turbinaria concoides*) were tested for antibacterial activity. A total of 14 bacteria were isolated, of which 6 were isolated from external tissue, while 8 from internal tissue. Results of an antagonistic test revealed that 7 isolates showed inhibitory activity against *Staphylococcus aureus* and only 1 isolate showed the inhibition against both *S. aureus* and *Escherichia coli*. Phenotypic and..." The browser's address bar shows the URL: <https://smujo.id/biodiv/authorDashboard/submission/6910>. The taskbar at the bottom shows the system time as 9:31 on 08/02/2023.

Dear Editor-in-Chief,

I herewith enclosed a research article,

Title:

Antibacterials Potential Symbiont Bacteria of Brown Algae (*Turbinaria Conoides*) Obtained from Indonesian waters

Author(s) name:

Niken Dharmayanti

Address

(Fill in your institution's name and address, your personal cellular phone and email)

Jakarta Fisheries Tehnical University, Pasar Minggu 12520, South Jakarta, Indonesia

Phone Number: 081385058734

Email: niken.stp@gmail.com

For possibility publication on the journal:

(fill in *Biodiversitas* or *Nusantara Bioscience* or mention the others)

Biodiversitas

Novelty:

Our research has identified antibacterial agents from endobionts associated with commonly-found brown seaweed in Indonesia. The anti-bacterial agents will have useful application in pharmaceuticals and other potential industrial application.

Statements:

This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.

Author(s) has been read and agree to the Ethical Guidelines.

List of five potential reviewers

(Fill in names of five potential reviewers that agree to review your manuscpt and their email addresses. He/she should have Scopus ID and come from different institution with the authors; and from at least three different countries)

Place and date:

Jakarta, 07 October 2020

Sincerely yours,

(fill in your name, no need scanned autograph)

Niken Dharmayanti

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Left: 1,8 cm, Right: 1,8 cm, Bottom: 2 cm, Header distance from edge: 1,25 cm, Footer distance from edge: 1,25 cm, Numbering: Restart each section

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Suppress line numbers

Formatted: Space After: 0 pt, Suppress line numbers

Antibacterials Potential Symbiont Bacteria of Brown Algae (*Turbinaria conoides*) Obtained From Banten Bay Serang District – Province Of Banten Indonesian Waters

Formatted: Space After: 12 pt

Formatted: Font: 16 pt

Formatted: Font: 10 pt, Bold

Formatted: Font: Bold

Formatted: Font: 10 pt, Bold

Formatted: Font: Bold

Formatted: Font: 10 pt, Bold

Formatted: Font: 8 pt

Formatted: Font: 8 pt

Formatted: Font: 9 pt

Formatted: Space After: 6 pt

Formatted: Font: 9 pt

Formatted: Font: 10 pt

Formatted: Centered, Indent: Left: 0,76 cm, No bullets or numbering

Formatted: Left: 1,8 cm, Right: 1,8 cm, Bottom: 2 cm, Header distance from edge: 1,25 cm, Footer distance from edge: 1,25 cm, Numbering: Restart each section

Commented [p1]: This is general information which is not directly related to the topic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: (Default) Times New Roman, 10 pt, Bold

Formatted: Normal, Centered, No bullets or numbering

Formatted: Font: 10 pt

Niken Dharmayanti, Aef Permadi, Yulianti H Sipahutar, Resmi Rumenta Siregar, Arpan Nasri Siregar, Randi Bokhi Salampessy, Sujulivani, Arma -Anti anti

Sekolah Tinggi Perikanan, Jakarta, Indonesia Study program of Fish Processing Technology, Jakarta Fisheries Technical University, Pasar Minggu 12520, South Jakarta, Indonesia

ABSTRACT

Brown seaweed has the potential to produce bioactive compounds. It has been shown that the bacteria associated with seaweed are involved in the production of metabolites associated with their host. Microbes may be present as a living symbiotic in association with other algae as epiphytes or endophytes (Kalaivani et al., 2016). In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were brought from Lima Island, Serang District, Banten Bay. Symbiont bacteria with tested for antibacterial activity, were isolated using the bioassay test method. A total of 14 isolates were isolated, 6 of which came from the outside external tissue, while 8 isolates came from the inside of the algae internal tissue. Through the antagonistic test, 7 isolates showed inhibitory activity against *Staphylococcus aureus* and 1 isolate bacteria showed the best inhibition against both *S.aureus* and *E.coli*. Selected isolates have the ability to inhibit *S.aureus* after diffusion paper disc tested. Phenotypic and genotypic identification showed that the species symbiont bacteria of *Turbinaria conoides* is was *Lactobacillus plantarum*.

Keywords: bioassay, antagonistic, diffusion paper disc, *Lactobacillus plantarum*.

1- INTRODUCTION

Seaweed is an algae that lives in the sea and belongs to the division of thallophyta. The classification of seaweed based on pigment content consists of 4 classes, namely green seaweed (*Chlorophyta*), red seaweed (*Rhodophyta*), brown seaweed (*Phaeophyta*) and blond seaweed (*Chrysochyta*) (Suparmi and Sahri, 2009). Indonesia is the largest producer of seaweed in the world (FAO 2016) cultured in nearshore coastal regions. In addition to its primary economical content, the secondary metabolite content of seaweed has the seaweeds potential of being a producer produce of diverse bioactive metabolites with vast activity as antibacterial, antiviral, antifungal and cytotoxic properties (Zainuddin and Malina, 2009 in Siregar et al., 2012). Bacteria usually live on a host by performing a mutually beneficial symbiosis (Sahara et al., 2013). It has been shown that the bacteria associated with seaweed as epiphytes or endophytes are involved in the production of metabolites that together with their host. Microbes can be present as a living symbiotic in union with various marine algae as epiphytes or endophytes (Sartika et al, 2014, Kalaivani et al., 2016). Symbiont bacteria isolates in algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of the form of symbiotic mutualism. Algae provide the places needed sites and nutrients the bacteria need, while the bacteria encourage growth and protect the algal surface against pathogens (Hollants et al., 2012 in Sartika et al, 2014). Seaweeds can secrete secondary metabolites with antibacterial properties (Burgess et al., 1999; Armstrong et al., 2001; Yanet et al., 2003 in Nofiani, 2005).

Brown algae and other types have been extensively analyzed for their antibacterial and antifungal activity (Bhakuni and Rawat, 2005). Previous research on brown algae *Sargassum* sp. Has been shown to have antimicrobial potential of bioactive proteins from bacteria symbiotic with it (Sartika et al, 2014) and the potential of bacterial algae. Here we evaluate the properties of the brown algae *Turbinaria conoides* in producing bioactive compounds in inhibiting including the inhibition of pathogenic bacteria Urinary Tract Infection (UTI) human pathogens (Kalaivani et al., 2016). *T. conoides* is a tropical marine algae widely distributed in coastal waters in SE Asia. We chose this alga following extensive trials on other common macroalgae including *Sargassum* spp. and *Eucheama cottonii*.

The study focused on identification of macroalgae found in the sampling sites, isolation symbionts of the *Turbinaria conoides*, selection of symbiotic bacteria isolates, testing of antibacterial potency by diffusion of paper discs, and identification of the phenotype and genotype *Turbinaria conoides* symbiont bacteria.

2- MATERIALS AND METHODS

Materials

The materials used in this research are Turbinaria conoides, pure cultures of *S.aureus*, pure culture of *E.coli*, aquades, nutrient broth (Oxoid), plate count agar (Oxoid), mueller hinton agar (Oxoid), sterile sea water, 70% alcohol, 95% alcohol, spiritus, crystal violet, iodine, safranin, immersion oil, carbolfucsin dyes, alcoholic acid, methylene blue, malachite green solution, safranin solution, filter paper, tissue, cotton, brown paper, paper disc, matches.

The equipments used are petri dishes, test tube, beaker, measuring cup, preparatory glass, measuring pipette (omnipipette), dropper pipette, tip pipette, micro pipette, mortar, tube rack, scales (vibra), inoculation loops, Spatula, bent glass, sterile plastic, magnetic stirrer, bunsen, hotplate (thermo scientific), scooter, microscope (olympus), scissors, tweezers, autoclave (kemoto scientific), incubator (memmert), oven (memmert), shaker (thermo Scientific), refrigerator (selecta), laminary air flow (telstar), ohp markers, elastic bands, centrifuge (eppendorf), eppendorf tube, vortex mixer (heidolph). Application GPS mobile phone

Methods Procedures

Sampling

Samples of *Turbinaria sp.* (about 1 kg wet weight) was were taken from Lima island (S: -6.001051o; E: 106.153804) around 1 kg for determination in the morning around 7 at low tide allows the position of algae 1 meter below the water on the sidelines of the reef directly dried in the bundle of the island. Sampling was continued at 16 o'clock when the low tide and taked 500 grams for antibacterial test and kept in the plastic pouch and immediately filled with seawater, with seaweed : water ratio of 1 : 2 until submerged, when it already arrived on Serang filled with oxygen 1 : 2 more air. The seaweed stayed in the plastic with oxygen from Serang until Jakarta for a night and started done in the laboratorium in the morning. Samples were maintained in fresh seawater for laboratory analyses within 24 hour, of collection.

Identification and Determination of Macroalgae

Dried *Turbinaria sp.* found in predetermined location or stations were recorded and identified by macroalgae type through algae base associated with observed macroalgae characteristics. The type of macroalgae used in this study was a genus of *Turbinaria sp.* The loeation is in the waters of the island of Lima, Banten Bay, Serang regency. The macroalgae determination used has done in LIPI Oceanography. Identification was performed on the method of form specification with reference to algae identification guidelines (Brigham et al., 2004; Lee, 2008)

Isolation of Symbiont Bacteria Producing Antibacterial Compounds

Surface of Algae- Epibionts were extracted from 15 grams of algae by rinsed-rinsin with 30 mL of sterile sea water. The rinse water is was put into incubated in 30 mL of nutrient broth medium then shaken by shaker at room temperature for 24 hours. Inside of algae: as many as Bioactive compound -15 grams of algae were rinsed with 30 mL of sterile sea water, were extracted by crushed-crushing 15 g of alga finely using mortar with a mortar and pestle with the addition of 15 ml of sterile seawater. The suspension is then fed was incubated into with 30 mL broth nutrient medium and shaken by shaker at room temperature for 24 hours.

After extraction process, the refreshed samples of in the 30 ml broth nutrient medium were diluted into 9 ml broth nutrient sterile by 10^{-1} up to 10^{-5} . Each dilutions was were grown on a plate count agar medium by incubate them at 37 °C for 2 x 24 hours. After incubating the petri dishes which were contain samples from each dilution, then the colonies bacteria from alga would appear. The colonies bacteria producing antimicrobial compounds are were characterized by a clear zone around the colonies. Furthermore, the colonies with stable inhibition zones were collected by and isolating themed on slant agar medium, with a clear code.

Selection of Symbiont Bacteria Isolates Antagonistically against Pathogenic Bacteria

To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria, a qualitative test was conducted directly by scratching or bottling round the isolates on the surface of the media that has been dispersed with test bacteria (*Eschericia coli* and *Staphylococcus aureus*). Then Media were incubated for 2 x 24-48 hours at 37 °C. Each scratching round of isolates was then marked by its a unique code.

Inhibition zones were read as the point determined as those showing clear zones around the colony of symbiont bacteria isolates. the more clear zone of isolates in inhibit for both *Escheriacia coli* and *Staphylococcus aureus* are the better their activity. Strains that showed maximum antagonistic effect againsts tested pathogens were choosed and marked by its eodeidentified. Isolates that These choosen isoate with appropriate code which was formed a clear zone or has with the a highest activity are waswere isolated and selected for further antibacterial testing by paper disc and identification of phenotype and genotpc testin.g.

Antibacterial Potential Testing of Symbiont Bacterial Isolate by Paper Disc Diffusion

Testing inhibitory the supernatant of symbiont bacteria on thefor inhibitory growth of *E.coli* and *S.aureus* was performed by the agar diffusion method (Hudzicki, 2009) REFERENCE. Supernatant was obtained by separating the filtrate and supernatant by centrifugation process centrifuge for 1 hour, temperature at (25 °C and 3000 rpm). Paper discs containing supernatant 40 µL and the negative control nutrient broth 40 µL which has allowed were Hried left for 1 hour to reduce the water excess. (dried), negative control nutrient broth 40 µL also has allowed for 1 hour to reduce the water excess

Commented [p2]: In the Materials and Methods you describe the methods (including materials used where relevant) to evaluate the antibacterial properties of isolates from *Turbinaria conoides*.

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (Australia)

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Space After: 0 pt

Formatted: Font: 10 pt

Formatted: Space After: 6 pt

Commented [p3]: Is this after the incubation in the broth? You need to provide more details on this.

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Space After: 6 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Commented [aa4]:

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Commented [p5]: This needs to be clarified.

Formatted: Font: 10 pt, Not Bold

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Not Bold

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Space After: 6 pt

Commented [p6]: You should refer to a standard test where possible. Here and throughout.

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Commented [aa7]:

Formatted: Font: 10 pt

Formatted: Font: 10 pt

(dried) and positive control chloramphenicol 0.01 mg/mL, were placed on the surface of the Mueller Hinton Agar A medium containing 1 mL test bacteria. Furthermore and incubated for 2 x 24 hours at 37 °C. The supernatant diffuses from the disc into the agar in decreasing amounts the further it is away from the disc. If the organism is killed or inhibited by both the supernatant and chloramphenicol as antibiotic positive control, there will be no growth in the immediate area around the disc, this is called the zone of inhibition. The zone sizes were compared up on a standardized to give a result of to assess bioactivity as sensitive, resistant, or intermediate, then it was observed and measured its in each case the resistance zone where shows no colonies growth with by a ruler was measured by using ruler to the nearest mm.

Commented [p8]: Spell out MHA

Commented [p9]: Are you drying 40 ul? Make clearer.

Formatted: Font: 10 pt

Identification of Phenotype and Genotype of Symbiotic Bacteria

In general, General bacterial identification was performed in accordance with the microbial analysis procedure in the laboratory (Lay, 1994 and identification keys from Cowan and Steel (1993)) by performing followed colony characteristic observations on liquid medium and solid medium, observing cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and test-Biochemistry test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase). The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *Turbinaria conoides* samples on the agar medium in pour plating. Observation of medium incubated with temperature 37°C was done at incubation time reached 24 hours and 48 hours. The data obtained from the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from the *Turbinaria conoides* seaweed. Determination of the type of bacteria was performed based on identification keys from Cowan and Steel (1993). Symbiotic bacteria species was determined by molecular testing.

Commented [aa10]:

Commented [aa11]: The meaning of resistance zone

Commented [p12]: Provide more details on this. What is a resistance zone? How is it measured?

Commented [aa13]:

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (Australia)

Formatted: Font: 10 pt

Formatted: Space After: 0 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Commented [p14]: I have moved this from the Discussion section. You need to provide more detail as to how you did this.

Formatted: Font: 10 pt

Formatted: Font: 10 pt

The DNA of the symbiotic bacteria isolates was amplified using primers 9F and 1541R. The DNA bands used were relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf German) with a first predenaturation at 94 ° C for 90 seconds, followed by 30 cycles consisting of denaturation at 95 ° C for 30 seconds, primary attachment at 50 ° C for 30 seconds and extension at 72 ° C for 90 seconds. After 30 cycles completed, followed by the elongation phase at 72 ° C for 5 min and cooling at 4 ° C for 20 min. Molecular identification was done through partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES method (Pitcher et al., 1989, Modified). PCR Amplification on 16S rDNA using Primer 9 F: 5' - AAG GAG GTG ATC CAG CC-3' and Primer 1541 R: 5' - GAG TTT GAT CCT GGC TCA G - 3' (White et al., 1990; O'Donnell, 1993). The analysis of nitrogen base sequence readings using was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data was were trimmed and assembled using the BioEdit program (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequencing data that has been were assembled in BLAST with genomic data that has been registered in DDBJ / DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>)

RESULTS RESULTS AND DISCUSSION

3.

The Result of Identification and Determination of Macroalga

The macroalgae observation area and the sample site obtained are determined based on the location coordinate point. The location of macroalgae observation S: 6.001051o; E: 106.153804o has morphology characteristic as Cylindrical rods, erect, rough, there are traces of branching. Holdfast is a small disc with radial expansion root. The branches rotate around the main trunk. Leaves are unity consisting of stalks and sheets. It named *Turbinaria conoides*.

Formatted: Font: 10 pt

Formatted: Indent: Left: 0,75 cm, No bullets or numbering



Formatted: Font: (Default) Times New Roman, 10 pt

The Result of Symbiont Bacteria Isolation

The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *Turbinaria conoides* samples on the agar medium in pour plating. Observation of medium incubated with temperature 37°C was done at incubation time reached 24 hours and 48 hours. When incubated, the individual microbial cells multiply so rapidly that within 18 to 24 hours a visible mass of cells is formed and is called a colony (Pelzear and Chan, 1986).



Figure 1. Growth of symbiont bacteria on agar medium

The grown from 40 samples consisted of the inside and outside of the algae, as many as 20 petri for 2 replications resulted in colonies with the inhibit zone of 14 colonies, 6 of which were from the outside epibionts, while the other 8 came from the inside of the algal tissue. The results of identification of colonies grown on mixed cultures can be seen in Table 21, and identification of isolates isolated into slant agar can be seen in Table 23.

Table 21. Macroscopic forms of bacterial colonies

No	Colony code	Morphology of colonies			
		Shape	Color	Edges	Elevation
1	TUL ² -A1-2	Round	White	Flat	Convex shiny
2	TUL ² -A2-2	Round	White	Flat	Convex shiny
3	TUL ² -A3-2	Round	White	Flat	Convex shiny
4	TUL ² -A4-2	Round	White	Flat	Convex shiny
5	TUL ² -B1-2	Round	White	Crooked	Convex shiny
6	TUL ² -B2-2	Round	White	Crooked	Convex shiny
7	TUD ⁴ -C1-2	Round	White	Flat	Convex shiny
8	TUD ⁴ -C2-2	Round	White	Flat	Convex shiny
9	TUD ² -D1-2	Round	White	Crooked	Convex shiny
10	TUD ² -D2-2	Round	White	Crooked	Convex shiny
11	TUD ² -D3-2	Round	White	Crooked	Convex shiny
12	TUD ² -D4-2	Round	White	Crooked	Convex shiny
13	TUD ⁵ -E-2	Round	White	Flat	Convex shiny
14	TUD ³ -F-2	Round	White	Flat	Convex shiny

Information:

- *The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
- ** The code of isolates (2), (4), (5), (3) states isolates obtained from the dilution
- *** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according to the best inhibition zone of each colony observed on the plate
- **** The code of number 2 identifies the isolate obtained from the second repeat

Table 23. Identification of the isolates on slant agar

No	Code of isolates	Solid medium	
		Shape	Color

Formatted: Font: (Default) Times New Roman, 10 pt
Formatted: Justified

Commented [p15]: Is this the number of samples tested?

Formatted: Font: 10 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted Table

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Left

Formatted Table

Formatted: Indent: Hanging: 1,46 cm

193 Bacterial isolates derived from the insidetissue showed have better activity better inhibition than baeterial_ isolates
194 derived from the surfaceepibionts. Inhibitory zone and diameter measurement results against S.aureus and E.coli can be
195 seen in Figure 34 and Table 34. Positive controls have a broader spectrum in inhibiting both types of test bacteria with 16.8
196 mm inhibition against S.aureus and 13.8 mm against E.coli. Chloramphenicol with a concentration of 0.03 mg on a paper
197 disc is highly active if its inhibition zone is more than 18 mm (Lay, 1994), while the dose of chloramphenicol (positive
198 control) used is lower at 0.01 mg, so it can be said that bacteria Test is sensitive to positive control. Negative control (NB
199 without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that a
200 supernatant still containing medium has no effect on the activity formed. From the stability of the measured inhibition zone,
201 the antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram positive
202 bacteria and are merely bacteriostatic for Gram negative bacteria. Paper disc with a supernatant applied to a Gram positive
203 bacterial plate indicates a stable clear zone even after a 48-hour incubation period. While against Gram negative bacteria,
204 around the disc paper shows the presence of inhibitory activity but gradually become turbid before the incubation period
205 reaches 24 hours.

206
207 According to Abubakar et al (2011) in Sartika (2014) the inner symbiotic bacteria generally have abundant
208 populations and are specific microbes because they directly interact with the bioactive compounds produced from within the
209 algae. While the symbiotic bacteria originating from the surface have a population that is less suspected because it requires
210 higher defense power to overcome the pathogens and predators that are around the algae.

211 Symbiont bacteria isolates in algae have abundant antimicrobial activity. The existence of the bacteria is suspected
212 as a result of the form of symbiotic mutualism. Algae provide the places and nutrients the bacteria need, while the bacteria
213 encourage growth and protect the algal surface against pathogens (Hollants et al., 2012 in Sartika, 2014).

214 The sequence of DNA sequencing results of the 16S rDNA encoding of symbiont bacteria isolates showed an accurate
215 score for species level with a similarity of > 99% of the sequences present in GenBank. Then the species homology of the
216 isolates tested was *Lactobacillus plantarum*. Classification of bacterial isolates are Bacteria; Firmicutes; Bacilli;
217 Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus plantarum.

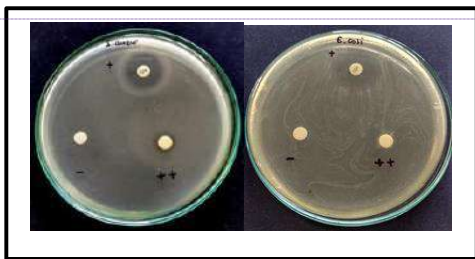
220 Discussion

221 Antibacterial Potential Testing of Symbiotic Bacteria Isolates by Discussion Paper Disc

222 Applications

223 The antibacterial compounds produced by symbiont bacteria isolates showed different inhibitory activity against both
224 tested bacteria *S.aureus* and *E.coli* with clear zone observations around the paper disc. Inhibitory zone and diameter diameter
225 measurement results against S.aureus and E.coli can be seen in Figure 4 and Table 4. According to Abubakar et al (2011)
226 in Sartika et al (2014) the inner symbiotic bacteria generally have abundant populations and are specific microbes because
227 they directly interact with the bioactive compounds produced from within the algae. While the symbiotic bacteria originating
228 from the surface have a population that is less suspected because it requires higher defense power to overcome the pathogens
229 and predators that are around the algae.

230
231 Positive controls have a broader spectrum in inhibiting both types of test bacteria with 16.8 mm
232 inhibition against S.aureus and 13.8 mm against E.coli. Chloramphenicol with a concentration of 0.03
233 mg on a paper disc is highly active if its inhibition zone is more than 18 mm (Lay, 1994), while the dose
234 of chloramphenicol (positive control) used is lower at 0.01 mg, so it can be said that bacteria Test is
235 sensitive to positive control. Negative control (NB without symbiotic bacterial inoculation) indicates
236 the absence of activity or inhibition zone, so it can be ascertained that a supernatant still containing
237 medium has no effect on the activity formed.



248 Figure 43. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

Commented [p16]: This is Discussion

Commented [p17]: This is also discussion

Commented [p18]: Moved to Introduction

Formatted: Space Before: 0 pt

Formatted: Indent: First line: 0 cm

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Space Before: 0 pt, Add space between paragraphs of the same style

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Commented [p19]: This is Discussion

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Commented [p20]: This is also discussion

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: English (Australia)

Formatted: Font: 9 pt

From the stability of the measured inhibition zone, in general the antibacterial properties of the supernatant produced by the symbiotic bacteria act as bactericidal against Gram positive bacteria and are merely bacteriostatic in Gram negative. Paper disc with a supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a 48 hour incubation period. While against Gram negative bacteria, around the disc paper shows the presence of inhibitory activity but gradually become turbid before the incubation period reaches 24 hours. Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Lay, 1994). Other factors that influence the ability of inhibitory inhibition are the concentration or intensity of antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of organic matter and the degree of acidity (pH) (Sulistijowati and Mile, 2015).

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 9 pt

Table 34. Results of measurement of inhibitory zone diameter of antibacterial compounds

Repetition	Diameter of zone inhibition (mm)					
	Gram positive			Gram negative		
	Symbiont bacterial (++)	Control (+)	Control (-)	Symbiont bacterial (++)	Control (+)	Control (-)
1	5,5	16	0	0	13,5	0
2	7,8	17,5	0	0	14	0
Average	6,7	16,8	0	0	13,8	0

Formatted Table

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

The area of the symptomatic supernatant inhibition zone of *S.aureus* is was 6.7 mm. According to Edrada (1998) in Kusumadewi (2004) a measured inhibition zone of less than 10 mm belongs to a very shows weak and very active activity if and strong activity if the the inhibition zone is greater than 15 mm. Testing of antibacterial activity of the symbiont bacteria supernatant obtained was still far from the results of the antibiotic activity of the tested compare chloramphenicol F control. This is because the antibacterial compound of the applied extracted symbiont bacteria is still was a supernatant with the containing secondary metabolites. it contains, but However, the test results have indicated the presence of provide clear evidence of antibacterial activity. Generally the chemical structure of metabolites from marine products is often different from the secondary differs from those of terrestrial origin metabolite of land (Gudbjarnason 1999 in Nofiani, 2005). Seawater contains an active inhibitor agent for Gram positive bacteria, according to Okami (1982) in in Nofiani (2005) that seawater contains an active inhibitor agent for organisms, seawater has the ability of inhibitors against Gram positive bacteria.

Formatted: Font: 10 pt

The activity of sea water inhibitor is not caused by faga or salinity but because there are antibacterial agents in seawater.

Formatted: Font: 10 pt

Based on the results of previous studies, most bacteria that live by associating with marine living creatures show great potential in secondary metabolite secretion with antibacterial properties (Burgess et al., 1999; Armstrong et al., 2001; Yanet et al., 2003 in Nofiani, 2005). Secondary metabolites are not used for growth and are formed from primary metabolites under stress conditions. Examples of secondary metabolites are antibiotics, pigments, toxins, ecologic and symbiotic competition effectors, pheromones, enzyme inhibitors, immunomodulating agents, antagonizing receptors and agonists, pesticides, antitumor agents, and promoters of plant and animal growth (Nofiani, 2005).

Commented [p21]: Moved to Introduction.

Formatted: Font: 10 pt

Identification of Phenotype and Genotype of Symbiont Bacteria

Formatted: Font: 10 pt

Known characteristics of the microscopic identification and biochemical tests of symbiont bacteria include the shape of a stem, non-acidic, non-spore forming, non-motile, aerobically grown, negative catalase, and positive carbohydrates test. Based on the identification keys of Cowan and Steel (1993) referring to the 12th digit in the table of indications which indicates there are five types of bacteria suspected of having similar characters namely *Brochothrix*, *Erysipelothrix*, *Lactobacillus*, *Arcanobacterium*, and *Arachnia*.

Formatted: Indent: First line: 0 cm

Commented [p22]: Not directly relevant

Formatted: Font: 10 pt

Based on phenotypic identification results through cell staining and biochemical testing, symbiont bacteria has were rod shaped, non-acidic, non-spore forming, non-motile, aerobically grown, negative catalase, and positive to carbohydrate

294 tests. In general, the identification of microscopically selected isolates showed specific characteristics possessed by
295 lactic acid bacteria (*Lactobacillus* spp.), such as round colonies, milky white, Gram positive with short stem cells, and
296 does without not forming endospores (Desniar 2012 in Saskia, 2014). The genus *Lactobacillus* can be isolated from
297 several different habitats, eg from milkfish intestine (Sulistijowati and Mile, 2015), bekasam products (Ingratubun et al.,
298 2013), up to coastal mangrove waters (Yahya et al., 2014).

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

299 The DNA of the symbiont bacteria isolates was amplified using primers 9F and 1541R. The DNA
300 bands used were relevant to the resulting PCR product of about 1400 base pairs. The sequence of DNA
301 sequencing results of the 16S rDNA encoding of symbiont bacteria isolates showed an accurate score
302 for species level with a similarity of $\geq 99\%$ of the sequences present in GenBank. Then the species
303 homology of the isolates tested was *Lactobacillus plantarum*. Classification of bacterial isolates are
304 Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus
305 plantarum.

Commented [p23]: Moved to Materials and Methods

Commented [p24]: Moved to Results

*Lactobacillus plantarum*_100%

```
GCTCAGGACGAACGCTGGCGGGCGTCCATACATGCAAGTCGAACGAACCTGGTATTGATTGGTCTGCATCATGATTACAT
TTGAGTGAGTGGCGAACTGGTGTAGTAACACGTGGGAAACCTGCCAGAAAGCGGGGATAACACCTGGAAACAGATGCTAATACCG
CATAACAACTTGACCGCATGTCCGAGCTTGAAGATGGCTTCGGCTATCACTTTGGATGGTCCCGCGCGTATTAGCTAGATG
GTGGGTAACGGCTACCATGGCAATGATACGTAGCGGCAAGCGTTGTCCGGATTTATTGGCGTAAAGCGAGCGCAGGCGGTTTTT
CTCCTACGGGAGGCGAGTGGGAATCTCCCAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTC
GGCTCGTAAAACCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAAGTATTGACGGTATTAAACCAGAAAGCCACGGCTA
ACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGCGTAAAGCGAGCGCAGGCGGTTTTT
AAGTCTGATGTGAAGCGCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAACTTGAAGTGCAGAAAGAGGACAGTGGAACTC
CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACCACAGTGGCGAAGCGCGTGTCTGGTCTGTAACGTACGCTGAGGCTC
GAAAGTATGGTAGCAACAGGATTAGATACCTGTAGTCCATACCGTAAACGATGAATGCTAAGTGTGGAGGGTTCCGGCCCT
TCAGTCTGCAGCTAACGCAATTAAGCATTCGGCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAAGCGGTGGAGCATGTGGTTAATTCGAAGCTACCGGAAGAACCTACCAAGTCTTGACATACTATGCAAATCTAAGAGATT
ACGCTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTGTGTCGTCAGCTCGTGCAGTGTGGTAAAGTCCCGCAACG
AGCCCAACCCCTTATTACAGTTGCCAGCATTAAAGTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGATG
ACGTAACATCATGACCCCTTATGACCTGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAAG
CTAATCTCTTAAAGCCATTCTCAGTTCCGATTGTAGGCTGCAACTCGCTACATGAAGTCCGGAATCGCTAGTAAATCGCGGATCAGC
ATGCCGCGGTAACGTTCCCGGGCCTGTACACACCGCCGTCACACCATGAGAGTTGTAACACCCAAAGTC
```

Formatted: Font: (Default) Arial, Bold

Formatted: Left

307 Figure 4. Sequens of 16S rDNA

Formatted: Font: 9 pt

Formatted: Centered

Formatted: Font: 10 pt

308 Genotypic identification of symbiont bacteria were using DNA encoding the 16S rDNA gene to determine genus and
309 strain. 16S rDNA can be used as a molecular marker for species definition because this molecule exists in every organism
310 with a function identical to all organisms. Data offor base sequence encoding gene of 16S rDNA can be seen in figure 4, it
311 shows that symbiont bacteria has accurate scores for species levels with a similarity 100% of the sequences present in
312 GenBank (Figure 4). The species homology of the tested isolate was *Lactobacillus plantarum*.

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

313 4. CONCLUSION

Formatted: Normal, Centered, Indent: First line: 1 cm, No bullets or numbering

Commented [p25]:

Commented [p26]: This is just repeating the results. Can be deleted.

314 Based on the survey results revealed that macroalgae were found in the Gulf of Banten, Serang district, Province of
315 Banten, named *Turbinaria conoides*. The result of the isolation of bacterial symbionts *Turbinaria conoides* isolates obtained
316 from colonies that produce inhibition zone in mix culture are 14 isolates, six of which came from the outside, while eight
317 other isolates came from the inside of the algae. Selection of 14 isolates through qualitative antagonist test showed that 7
318 isolates showed inhibitory activity against *S.aureus* and 2 isolates showed the best inhibition against *E.coli*. In general,
319 isolates with code TUD4 C2-2 were selected isolates and showed a better potential for *S.aureus* through diffusion test of
320 paper disc. Through molecular (DNA) test it was known that the symbiont species of *Turbinaria conoides* was *Lactobacillus*
321 *plantarum*.

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: English (Australia)

Formatted: Font: 10 pt

Formatted: Centered

Formatted: Font: 8 pt

Formatted: Justified

Formatted: Font: 8 pt

Formatted: Justified

Formatted: Font: 8 pt, Italic

Formatted: Font: 8 pt

322 *Turbinaria conoides* is one of macroalga which is find atcommonly found in the gulf of Banten, Serang district,
323 province of Banten. Based on the results of this This research known shows that symbiont bacteria *Lactobacillus plantarum*
324 could living in the macroalga as endophytic are endophytic and potentially useful as an antibacterial agent against common
325 pathogens. The symbiont bacteria produced bioactive compound which was inhibited Gram positif
326 phatogen bacteria *Staphylococcus aureus*.

327 REFERENCES

328 Phakuni, D.S dan Rawat, D.S. 2005. *Bioactive Marine Natural Products*. ISBN 1-4020-3472-5 (HB). Published by Springer New York 10013, USA and
329 Anamaya Publishers, New Delhi, India.
330 Cowan, S-T and Steel, K.J. 1993. *Manual for the Identification of Medical Bacteria*. 3rd Edition. University of Cambridge. UK.
331 Dwidjoseputro, D. 1981. *Dasar-dasar Mikrobiologi*. Cetakan ke-5. Djambatan, 1981: Jakarta.
332
333 Hudzicki. 2009. *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*. ASM Microbelibrary, American Society for Microbiology. [http://www.
334 microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauerdisk-diffusion-susceptibility-test-protocol..](http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauerdisk-diffusion-susceptibility-test-protocol..) (7/04/2014)

Formatted: Font: 8 pt, Italic

Formatted: Font: 8 pt

335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366

Ingratubun, J. A., Ijong, F. G., dan Onibala, H. 2013. *Isolasi dan Identifikasi Bakteri Asam Laktat pada Bakasang sebagai Starter Mikroba Produk Fermentasi*. Jurnal. Aquatic Science & Management, Edisi Khusus 1, 48-56. Pascasarjana, Universitas Sam Ratulangi.

Kalaivani, G., Hemalatha, N., dan Poongothai. 2016. *Screening Of Marine Brown Algae Associated Potential Bacteria Producing Antagonistic Bioactive Compounds Against Uti Pathogens*. International Journal of Pharma and Bio Sciences 2016 April; 7(2): (B) 395 – 405. India.

Kusumadewi, R. 2004. *Penapisan Awal Senyawa Bioaktif Antibakteri dari Melati Laut (Clerodendrum inerme)*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor: Bogor.

Lay, B.-W. 1994. *Analisis Mikroba di Laboratorium*. PT. Raja Grafindo Persada: Jakarta

Lukman, J.B., Dwyana Z., Raya, I., Priosambodo, D. 2015. *Efektivitas Ekstrak Alga Eucheuma Cottonii, Turbinaria Decurrens, dan Ulva Reticulata Sebagai Antimikroba terhadap Streptococcus Mutans*. Jurnal. Jurusan Biologi-FMIPA Universitas Hasanuddin: Makassar.

Nofiani, R. 2005. *Urgensi dan Mekanisme Biosintesis Metabolit Sekunder Mikroba Laut*. Jurnal Natur Indonesia 10 (2), April 2008: 120-125.

O'Donnell K. 1993. *Fusarium and its near relatives*. In: Reynolds DR & Taylor JW (Eds) *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics* (pp 225–233). CAB International, Wallingford, UK.

Pitcher DG, Saunders NA, Owen RJ. 1989. *Rapid extraction of bacterial genomic DNA with guanidium thiocyanate*. Letters in Applied Microbiology 1989. 8: 151-156.

Pelezar, M.J dan Chan, E.C.S. 1986. *Dasar-dasar Mikrobiologi*. Diterjemahkan oleh Ratnasari, dkk. Edisi 1. UI Press, Jakarta

Sahara, F. N. I., Radjasa, O., K., dan Supriyantini, E. 2013. *Identifikasi Pigmen Karotenoid pada Bakteri Symbion Rumpul Laut Kappahyeus alvarezii*. Journal Of Marine Research Volume 2, Nomor 3, Tahun 2013, Halaman 58-67. Online di: <http://ejournal-s1.undip.ac.id/index.php/jmr>

Sartika, Ahmad, A., dan Natsir, H. 2014. *Potensi Antimikroba Protein Bioaktif dari Bakteri Symbion Alga Coklat Sargassum sp. Asal Perairan Pulau Laelae*. Jurnal: FMIPA Universitas Hasanuddin. Makassar.

Saskia, A. 2014. *Pengembangan Kultur Kering Bakteri Lactobacillus plantarum (SK5) asal Bekasam sebagai Kandidat Probiotik dengan Teknik Pengeringan Beku*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor: Bogor.

Siregar, A.-F., Sabdono, A., dan Pringgienis, D. 2012. *Potensi Antibakteri Ekstrak Rumpul Laut Terhadap Bakteri Penyakit Kulit Pseudomonas aeruginosa, Staphylococcus epidermidis, dan Micrococcus*. Journal Of Marine Research. Volume 1, Nomor 2, Tahun 2012, Halaman 152-160.

Sulistijowati, R dan Mile, L. 2015. *Efektivitas Penghambatan Filtrat Asam Laktat Lactobacillus Sp. Hasil Isolasi Dari Usus Ikan Bandeng (Chanos chanos) Terhadap Bakteri Patogen*. Fakultas Perikanan dan Ilmu Kelautan Universitas Negeri Gorontalo.

Suparmi dan Sahri, A. 2009. *Kajian Pemanfaatan Sumber Daya Rumpul Laut dari Aspek Industri Dan Kesehatan*. Jurnal: Sultan Agung Vol XLIV No. 96: 18. Fakultas Kedokteran Universitas Islam Sultan Agung.

White TJ, Bruns T, Lee, S. and Taylor JW. 1990. *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. Pp. 315-322. In: PCR Protocols: A guide to Methods and Applications. Academic Press, Inc., New York.

Yahya, Nursyam, H., Risjani, Y., dan Soemarno. 2014. *Karakteristik Bakteri di Perairan Mangrove Pesisir Kraton Pasuruan*. Jurnal. Ilmu Kelautan Maret 2014. Vol. 19(1):35-42. Pasca Sarjana Fakultas Pertanian, Universitas Brawijaya.

- Formatted: Justified
- Formatted: Font: 8 pt, Not Bold
- Formatted: Font: 8 pt
- Formatted: Indent: Left: 0 cm, Hanging: 2,25 cm
- Formatted: Font: 8 pt
- Formatted: Font: (Default) Times New Roman, 8 pt
- Formatted: Normal, Indent: Left: 0 cm, Hanging: 1,27 cm, Line spacing: 1,5 lines
- Formatted: Font: 8 pt
- Formatted: Font: 8 pt
- Formatted: Font: 8 pt, Not Bold
- Formatted: Font: 8 pt, Not Bold
- Formatted: Font: 8 pt, Not Bold
- Formatted: Font: 8 pt
- Formatted: Indent: Left: 0 cm, Hanging: 2,25 cm
- Formatted: Left: 1,8 cm, Right: 1,8 cm, Bottom: 2 cm

Dear Editor-in-Chief,

I herewith enclosed a research article,

Title:

Antibacterials Potential Symbiont Bacteria of Brown Algae (*Turbinaria Conoides*) Obtained from Indonesian waters

Author(s) name:

Niken Dharmayanti

Address

(Fill in your institution's name and address, your personal cellular phone and email)

Jakarta Fisheries Tehnical University, Pasar Minggu 12520, South Jakarta, Indonesia

Phone Number: 081385058734

Email: niken.stp@gmail.com

For possibility publication on the journal:

(fill in *Biodiversitas* or *Nusantara Bioscience* or mention the others)

Biodiversitas

Novelty:

Our research has identified antibacterial agents from endobionts associated with commonly-found brown seaweed in Indonesia. The anti-bacterial agents will have useful application in pharmaceuticals and other potential industrial application.

Statements:

This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.

Author(s) has been read and agree to the Ethical Guidelines.

List of five potential reviewers

(Fill in names of five potential reviewers that agree to review your manuscpt and their email addresses. He/she should have Scopus ID and come from different institution with the authors; and from at least three different countries)

Place and date:

Jakarta, 07 October 2020

Sincerely yours,

(fill in your name, no need scanned autograph)

Niken Dharmayanti

Commented [N01]:

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Left: 1,8 cm, Right: 1,8 cm, Bottom: 2 cm, Header distance from edge: 1,25 cm, Footer distance from edge: 1,25 cm, Numbering: Restart each section

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Space After: 0 pt, Line spacing: single, Suppress line numbers

Formatted: Suppress line numbers

Formatted: Space After: 0 pt, Suppress line numbers

Antibacterials Potential Symbiont Bacteria of Brown Algae (*Turbinaria conoides*) Obtained From Banten Bay Serang District – Province Of Banten Indonesian Waters

Niken Dharmayanti, Aef Permadi, Arma Anti, Resmi Rumenta Siregar, Yuliati H Sipahutar, Resmi Rumenta Siregar, Arpan Nasri Siregar, Yuliati H Sipahutar, Aef Permadi, Randi Bokhi Salampessy, Sujulivani, Arpan Nasri Siregar, Randi Bokhi Salampessy, Sujulivani, Siti Zachro Nurbani, Heni Budi Purnamasari, Arma Anti anti

Sekolah Tinggi Perikanan, Jakarta, Indonesia Study program Program of Fish Processing Technology, Jakarta Technical University of Fisheries Technical University, Pasar Minggu 12520, South Jakarta, Indonesia

ABSTRACT

Brown seaweed has the potential to produce bioactive compounds. It has been shown that the bacteria associated with seaweed are involved in the production of metabolites associated with their host. Microbes may be present as a living symbiotic in association with other algae as epiphytes or endophytes (Kalaivani et al., 2016). In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were brought from Lima Island, Serang District, Banten Bay. Symbiont bacteria with tested for antibacterial activity, were isolated using the bioassay test method. A total of 14 isolates were isolated, 6 of which came from the outside external tissue, while 8 isolates came from the inside of the algae internal tissue. Through the antagonistic test, 7 isolates showed inhibitory activity against *Staphylococcus aureus* and 1 isolate bacteria showed the best inhibition against both *S.aureus* and *E.coli*. Selected isolates have the ability to inhibit *S.aureus* after diffusion paper disc tested. Phenotypic and genotypic identification showed that the species symbiont bacteria of *Turbinaria conoides* is was *Lactobacillus plantarum*.

Keywords: bioassay, antagonistic, diffusion paper disc, *Lactobacillus plantarum*.

INTRODUCTION

Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse and unique compounds (Bahare S et al. 2019). Many substances obtained from seaweed, such as alginates, carrageenan, and agar have been used for decades in traditional medicine, pharmacology, and food (Andrea GZ et al. 2019). Other compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory and antifouling activity. Therefore, seaweed can provide promising bioactives that can be used in the treatment of human diseases, or new antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. Much attention has been paid to developing innovative projects for pharmaceuticals, seaweed applications, especially in the design of new antimicrobial drugs. Research for the identification of promising algal species, standardization of analytical methods, isolation of compounds through integrated fractionation of bioassays, detailed chemical characterization and evaluation of their safety, evaluation of synergistic effects between components, and efforts to improve yields, and lowering extraction costs, is needed (Marie JP et al. 2016).

Seaweed is an algae that lives in the sea and belongs to the division of thallophyta. The classification of seaweed based on pigment content consists of 4 classes, namely green seaweed (*Chlorophyta*), red seaweed (*Rhodophyta*), brown seaweed (*Phaeophyta*) and blond seaweed (*Chrysophyta*) (Suparmi and Sahri, 2009). Indonesia is the largest producer of seaweed in the world (FAO 2016) cultured in nearshore coastal regions. In addition to its primary economical content, the secondary metabolite content of seaweed has the Seaweeds potential of being a producer produce of diverse bioactive metabolites with vast activity as antibacterial, antiviral, antifungal and cytotoxic properties (Zainuddin and Malina, 2009 in Siregar et al., 2012). Bacteria usually live on a host by performing a mutually beneficial symbiosis (Sahara et al., 2013). It has been shown that the bacteria associated with seaweed as epiphytes or endophytes are involved in the production of metabolites that together with their host. Microbes can be present as a living symbiotic in union with various marine algae as epiphytes or endophytes (Alessandro B et al. 2017 Sartika et al., 2014, Kalaivani et al., 2016). Symbiont bacteria isolates in algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of the form of symbiotic mutualism. Algae provide the places needed sites and nutrients the bacteria need, while the bacteria encourage growth and protect the algal surface against pathogens (Mark LW et al. 2016 Hollants et al., 2012 in Sartika et al., 2014). Seaweeds can secrete secondary metabolites with antibacterial properties (Burgess et al., 1999; Armstrong et al., 2001; Yanet et al., 2003 in Nofiani, 2005 (Emer S and Nissreen AG 2016). The recent scientific trends focus on search of phytochemicals from marine algae due to their numerous health-promoting effects, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011)

Formatted: Space After: 12 pt

Formatted: Font: 16 pt

Formatted: Font: 10 pt, Bold

Formatted: Font: 10 pt, Bold

Formatted: Font: 10 pt, Bold

Formatted: Font: 8 pt

Formatted: Font: 8 pt

Formatted: Font: 9 pt

Formatted: Space After: 6 pt

Formatted: Font: 9 pt

Formatted: Font: 10 pt

Formatted: Centered, Indent: Left: 0,76 cm, No bullets or numbering

Formatted: Left: 1,8 cm, Right: 1,8 cm, Bottom: 2 cm, Header distance from edge: 1,25 cm, Footer distance from edge: 1,25 cm, Numbering: Restart each section

Formatted: Font color: Text 1

Commented [p2]: This is general information which is not directly related to the topic

Formatted: Font: 10 pt

Formatted: List Paragraph, Space Before: 0 pt, Add space between paragraphs of the same style

Formatted: Font color: Text 1

Formatted: Font: 10 pt

30 *Tubunaria conoides* belongs to the family of Sargassaceae (brown algae) is coming under the order of Fucales. It has
31 traditionally been used for children's fever, as a fertilizer, insect repellent, pesticide and antibacterialcidal (Arumugama P et
32 al. 2017)

33
34 Brown algae and other types have been extensively analyzed for their antibacterial and antifungal activity (Bhakuni
35 and Rawat, 2005). Previous research on brown algae *Sargassum sp.* Has been shown to have antimicrobial potential of
36 bioactive proteins from bacteria symbiotic with it (Sartika et al, 2014) and the potential of bacterial algae. Here we evaluate
37 the properties of the brown algae *Turbinaria conoides* in producing bioactive compounds in-inhibitingincluding the
38 inhibition of pathogenic bacteria Urinary Tract Infection (UTI) human pathogens (Kalaivani et al., 2016). *T. conoides* is a
39 tropical marine alga widely distributed in coastal waters in SE Asia. We chose this alga following extensive trials on other
40 common macroalgae including *Sargassum spp.* and *Eucheama cottonii*.

41 The study focused on identification of macroalgae found in the sampling sites, isolation symbionts
42 of the *Turbinaria conoides*, selection of symbiotic bacteria isolates, testing of antibacterial potency by
43 diffusion of paper discs, and identification of the phenotype and genotype *Turbinaria conoides*
44 symbiont bacteria.

45 46 47 2. MATERIALS AND METHODS

48 Materials

49 The materials used in this research are *Turbinaria conoides*., pure cultures of *S.aureus*, pure culture of *E.coli*,
50 aquades, nutrient broth (Oxoid), plate count agar (Oxoid), mueller hinton agar (Oxoid), sterile sea water, 70% alcohol, 95%
51 alcohol, spiritus, crystal violet, iodine, safranin, immersion oil, carbolfucsin dyes, alcoholic acid, methylene blue, malachite
52 green solution, safranin solution, filter paper, tissue, cotton, brown paper, paper disc, matches.

53 The equipments used are petri dishes, test tube, beaker, measuring cup, preparatory glass, measuring pipette
54 (omnipipette), dropper pipette, tip pipette, micro pipette, mortar, tube rack, scales (vibra), inoculation loops, Spatula, bent
55 glass, sterile plastic, magnetic stirrer, bunsen, hotplate (thermo scientific), scooter, microscope (olympus), scissors, tweezers,
56 autoclave (kemoto scientific), incubator (memmert), oven (memmert), shaker (thermo Scientific), refrigerator (selecta),
57 laminary air flow (telstar), ohp markers, elastic bands, centrifuge (eppendorf), eppendorf tube, vortex mixer
58 (heidolph). Application GPS mobile phone

60 Methods/Procedures

61 Sampling

62 Samples of *Turbinaria sp.* (about 1 kg wet weight) was were taken from Lima island (S: -6.0010516; E:
63 106.153804) around 1 kg for determination in the morning around 7 at low tide allows the position of algae 1 meter below
64 the water on the sidelines of the reef directly dried in the bundle of the island. Sampling was continued at 16 o'clock when
65 the low tide and taked 500 grams for antibacterial test and kept in the plastic pouch and immediately filled with seawater,
66 with seaweed : water ratio of 1 : 2 until submerged, when it already arrived on Serang filled with oxygen 1 : 2 more air. The
67 seaweed stayed in the plastic with oxygen from Serang until Jakarta for a night and started done in the laboratorium in the
68 morning. Samples were maintained in fresh seawater for laboratory analyses within 24 hour of collection.

69 Identification and Determination of Macroalgae

70 Dried *Turbinaria sp.* found in predetermined location or stations were recorded and identified by macroalgae type
71 through algae base associated with observed macroalgae characteristics. The type of macroalgae used in this study was a
72 genus of *Turbinaria sp.* The location is in the waters of the island of Lima, Banten Bay, Serang regency. The macroalgae
73 determination used has done in LIPI Oceanography. Identification was performed on the method of form specification with
74 reference to algae identification guidelines (Brigham et al., 2004; Lee, 2008)

75 Isolation of Symbiont Bacteria Producing Antibacterial Compounds

76 Surface of Algae-Epibionts were extracted from 15 grams of algae by rinsed-rinsin with 30 mL of sterile sea water.
77 The rinse water is was put into incubated in 30 mL of nutrient broth medium then shaken by shaker at room temperature for
78 24 hours. Inside of algae: as many as Bioactive compound -15 grams of algae were rinsed with 30 mL of sterile sea water, were
79 extracted by crushed-crushing 15 g of alga finely using mortar with a mortar and pestle with the addition of 15 ml of sterile
80 seawater. The suspension is then fed was incubated into with 30 mL broth nutrient medium and shaken by shaker at room
81 temperature for 24 hours.

82 After extraction process, the refreshed samples of-in the 30 ml broth nutrient medium were diluted into 9 ml broth
83 nutrient sterile by 10^{-1} up to 10^{-5} . Each dilutions was-were grown on a plate count agar medium by incubate them at 37 °C
84 for 2 x 24 hours. After incubating the petri dishes which were contain samples from each dilution, then the colonies bacteria
85 from alga would appear. The colonies bacteria producing antimicrobial compounds are-were characterized by a clear zone

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: (Default) Times New Roman, 10 pt, Bold

Formatted: Normal, Centered, No bullets or numbering

Formatted: Font: 10 pt

Commented [p3]: In the Materials and Methods you describe the methods (including materials used where relevant) to evaluate the antibacterial properties of isolates from *Turbinaria conoides*.

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (Australia)

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Space After: 0 pt

Formatted: Font: 10 pt

Formatted: Space After: 6 pt

86 around the colonies. Furthermore, the colonies with stable inhibition zones were collected by and isolating themed on
87 slant agar medium, with a clear code.

88 Selection of Symbiotic Bacteria Isolates Antagonistically against Pathogenic Bacteria

91 To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria, a qualitative test was
92 conducted directly by scratching or bottling round the isolates on the surface of the media that has been dispersed with test
93 bacteria (*Escherichia coli* and *Staphylococcus aureus*). Then Media were incubated for 2 x 24-48 hours at 37 °C. Each
94 scratching round of isolates was then marked by its a unique code.

95 Inhibition zones were read as the point determined as those showing clear zones around the colony of symbiont
96 bacteria isolates. the more clear zone of isolates in inhibit for both Escheriacia coli and Staphylococcus aureus are the better
97 their activity. Strains that showed maximum antagonistic effect againsts tested pathogens were choosed and marked by its
98 code identified. Isolates that These choosen isoate with appropriate code which was formed a clear zone or has with the a
99 highest activity are waswere isolated and selected for - further antibacterial testing by paper disc and identification of
100 phenotype and genotype testin.g.

101 Antibacterial Potential Testing of Symbiotic Bacterial Isolate by Paper Disc Diffusion

102 Testing inhibitory the supernatant of symbiotic bacteria on thefor inhibitory growth of *E.coli* and *S.aureus* was
103 performed by the agar diffusion method (Hudzicki, 2009; Grela E et al. 2018) REFERENCE. Supernatant was obtained by
104 separating the filtrate and supernatant by centrifugation process centrifuge for 1 hour, temperature at (25 °C and 3000 rpm).
105 Paper discs containing supernatant 40 µL and the negative control nutrient broth 40 µL which has allowed were dried left
106 for 1 hour to reduce the water excess (dried), negative control nutrient broth 40 µL also has allowed for 1 hour to reduce
107 the water excess (dried) and positive control chloramphenicol 0.01 mg/mL, were placed on the surface of the Mueller Hinton
108 Agar A medium containing 1 mL test bacteria. Furthermore and incubated for 2 x 24-48 hours at 37 °C. The supernatant
109 diffuses from the disc into the agar in decreasing amounts the further it is away from the disc. If the organism is killed or
110 inhibited by both the supernatant and chloramphenicol as antibiotic positive control, there will be no growth in the immediate
111 area around the disc, this is called the zone of inhibition. The zone sizes awere compared up on a standardized to give a
112 result of to assess bioactivity as sensitive, resistant, or intermediate, te then It was observed and measured its in each case
113 the resistance zone - where shows no colonies growth with by a ruler was measured by using ruler to the nearest mm.

114 Identification of Phenotype and Genotype of Symbiotic Bacteria

116 In general, General bacterial identification was performed in accordance with the microbial analysis procedure in
117 the laboratory (Phumudzo T, 2013; Lay, 1994 and identification keys from Cowan and Steel (1993)) by performing followed
118 colony characteristic observations on liquid medium and solid medium, observing cell morphology (gram staining, spore
119 staining, and Ziehl-Neelsen staining), and test Biochemistry test (motility, gelatin hydrolysis, citrate, urease, carbohydrates,
120 and catalase). The initial selection of isolates from mixed cultures was carried out after enrichment and planting of
121 Turbinaria conoides samples on the agar medium in pour plating. Observation of medium incubated with temperature 37°C
122 was done at incubation time reached 24 hours and 48 hours. The data obtained from the bacterial isolate characterization
123 were used to estimate the type of symbiotic bacteria isolated from the Turbinaria conoides seaweed. Determination of the
124 type of bacteria was performed based on identification keys from Cowan and Steel (1993). Symbiotic bacteria species was
125 determined by molecular testing.

127 The DNA of the symbiotic bacteria isolates was amplified using primers 9F and 1541R. The DNA bands used were
128 relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf German)
129 with a first predenaturation at 94 ° C for 90 seconds, followed by 30 cycles consisting of denaturation at 95 ° C for 30
130 seconds, primary attachment at 50 ° C for 30 seconds and extension at 72 ° C for 90 seconds. After 30 cycles completed,
131 followed by the elongation phase at 72 ° C for 5 min and cooling at 4 ° C for 20 min. Molecular identification was done
132 through partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES method (Pitcher et al., 1989,
133 Modified). PCR Amplification on 16S rDNA using Primer 9 F: 5' - AAG GAG GTG ATC CAG CC-3' and Primer 1541
134 R: 5' - GAG TTT GAT CCT GGC TCA G - 3' (White et al., 1990; O'Donnell, 1993). The analysis of nitrogen base sequence
135 readings using was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied
136 Biosystems). The next sequenced raw data waswere trimmed and assembled using the BioEdit program
137 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequencing data that has beenwere assembled in BLAST with genomic
138 data that has been registered in DDBJ / DNA Data Bank of Japan (http://blast.ddbj.nig.ac.jp/)

Commented [p4]: Is this after the incubation in the broth? You need to provide more details on this.

Formatted: Font: 10 pt

Formatted

Formatted: Space After: 6 pt

Formatted

Commented [aa5]:

Formatted

Commented [p6]: This needs to be clarified.

Formatted: Font: 10 pt

Formatted: Space After: 6 pt

Commented [p7]: You should refer to a standard test where possible. Here and throughout.

Formatted: Font: 10 pt

Formatted

Commented [N08]:

Formatted: Font: 10 pt

Commented [aa9]:

Formatted

Commented [p10]: Spell out MHA

Commented [p11]: Are you drying 40 ul? Make clearer.

Formatted: Font: 10 pt

Commented [aa12]:

Commented [aa13]: The meaing of resistance zone

Commented [N014]:

Commented [aa15]:

Commented [N016]:

Formatted: Font: 10 pt

Formatted

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (Australia)

Formatted: Font: 10 pt

Formatted: Space After: 0 pt

Formatted

Commented [p17]: I have moved this from the Discussion section. You need to provide more detail as to how you did this.

Formatted

RESULTS RESULTS AND DISCUSSION

3.

The Result of Identification and Determination of Macroalga

The macroalgae observation area and the sample site obtained are determined based on the location coordinate point. The location of macroalgae observation S: 6.001051o; E: 106.153804o has morphology characteristic as Cylindrical rods, erect, rough, there are traces of branching. Holdfast is a small disc with radial expansion root. The branches rotate around the main trunk. Leaves are unity consisting of stalks and sheets. It named *Turbinaria conoides*.



Turbinaria conoides

The Result of Symbiont Bacteria Isolation

The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *Turbinaria conoides* samples on the agar medium in pour plating. Observation of medium incubated with temperature 37°C was done at incubation time reached 24 hours and 48 hours. When incubated, the individual microbial cells multiply so rapidly that within 18 to 24 hours a visible mass of cells is formed and is called a colony (Pelzear and Chan, 1986).



Figure 1. Growth of symbiont bacteria on agar medium

The grown from 40 samples consisted of the inside and outside of the algae, as many as 20 petri for 2 replications resulted in colonies with the inhibit zone of 14 colonies, 6 of which were from the outside epibionts, while the other 8 came from the inside of the algal tissue. The results of identification of colonies grown on mixed cultures can be seen in Table 21, and identification of isolates isolated into slant agar can be seen in Table 23.

Table 21. Macroscopic forms of bacterial colonies

No	Colony code	Morphology of colonies			
		Shape	Color	Edges	Elevation

Formatted: Font: 10 pt

Formatted: Indent: Left: 0,75 cm, No bullets or numbering

Formatted: Font: (Default) Times New Roman, 10 pt

Formatted: Font: (Default) Times New Roman, 10 pt

Formatted: Justified

Commented [p18]: Is this the number of samples tested?

Formatted: Font: 10 pt

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted Table

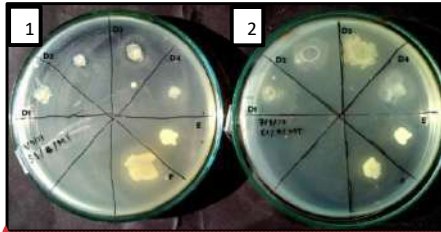


Figure 23. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

Based on the results of the direct challenge test, 7 bacterial isolates from 14 isolates tested showed inhibitory activity against *S.aureus* and only 2 of the 7 isolates had inhibitory activity against *E.coli*. The isolate codes that have inhibitory zones against *S.aureus* bacteria are TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2, whereas TUD4-C1-2, and TUD4-C2-2 have showed inhibition zones against both pathogenic bacteria being tested but the inhibitory activity against *E.coli* is was not as good as its inhibition against *S.aureus*.

Symbiotic bacterial isolates with a specific code that has a resistor zone are re-selected by looking at the best and largest clear zone. From the observation result, it was determined that isolates with code TUD4 C2 2 were isolates which had the best inhibition zone. Based on the code given, it is known that this isolate was obtained from the algae's inner sample, at 10⁻⁴ dilution, the second colony of the isolated third plate, and a colony obtained in the second repetition. Isolates with a specific code that has a showing inhibition zone were re-selected by looking at the best and largest clear zone. Isolates with code TUD4-C2-2 were isolates which had the best inhibition zone. From the observation result, it was determined that isolates with code TUD4 C2 2 were isolates which had the best inhibition zone. Based on the code given, it is known that this isolate was obtained from the algae's inner sample, at 10⁻⁴ dilution, the second colony of the isolated third plate, and a colony obtained in the second repetition.

Bacterial isolates derived from the inside tissue showed have better activity better inhibition than bacterial isolates derived from the surface epibionts. Inhibitory zone and diameter measurement results against *S.aureus* and *E.coli* can be seen in Figure 34 and Table 34. Positive controls have a broader spectrum in inhibiting both types of test bacteria with 16.8 mm inhibition against *S.aureus* and 13.8 mm against *E.coli*. Chloramphenicol with a concentration of 0.03 mg on a paper disc is highly active if its inhibition zone is more than 18 mm (Mounyr B et al., 1994-2016), while the dose of chloramphenicol (positive control) used is lower at 0.01 mg, so it can be said that bacteria Test is sensitive to positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that a supernatant still containing medium has no effect on the activity formed. From the stability of the measured inhibition zone, the antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram positive bacteria and are merely bacteriostatic for Gram negative bacteria. Paper disc with a supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a 48-hour incubation period. While against Gram negative bacteria, around the disc paper shows the presence of inhibitory activity but gradually become turbid before the incubation period reaches 24 hours.

According to Abubakar et al (2011) in Sartika (2014) the inner symbiotic bacteria generally have abundant populations and are specific microbes because they directly interact with the bioactive compounds produced from within the algae. While the symbiotic bacteria originating from the surface have a population that is less suspected because it requires higher defense power to overcome the pathogens and predators that are around the algae.

Symbiotic bacteria isolates in algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of the form of symbiotic mutualism. Algae provide the places and nutrients the bacteria need, while the bacteria encourage growth and protect the algal surface against pathogens (Hollants et al., 2012 in Sartika, 2014).

The sequence of DNA sequencing results of the 16S rDNA encoding of symbiont bacteria isolates showed an accurate score for species level with a similarity of > 99% of the sequences present in GenBank, Then the species homology of the isolates tested was *Lactobacillus plantarum*. Classification of bacterial isolates are Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus plantarum.

Discussion

Antibacterial Potential Testing of Symbiont Bacteria Isolates by Discussion Paper Disc

Applications

The antibacterial compounds produced by symbiont bacteria isolates showed different inhibitory activity against both tested bacteria *S.aureus* and *E.coli* with clear zone observations around the paper disc. Inhibitory zone and diameter diameter measurement results against *S.aureus* and *E.coli* can be seen in Figure 4 and Table 4. According to Irma ESM Abubakar et al (2011) in Sartika et al (2014) the inner symbiotic bacteria generally have abundant populations and are specific microbes

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Not Highlight

Formatted: Font: 10 pt

Formatted: Font color: Text 1

Formatted: Font: 10 pt

Commented [p19]: This is Discussion

Commented [p20]: This is also discussion

Commented [p21]: Moved to Introduction

Formatted: Space Before: 0 pt

Formatted: Indent: First line: 0 cm

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Formatted: Font: 10 pt

because they directly interact with the bioactive compounds produced from within the algae. While the symbiotic bacteria originating from the surface have a population that is less suspected because it requires higher defense power to overcome the pathogens and predators that are around the algae.

Positive control inhibition against *S. aureus* using a paper disc impregnated with chloramphenicol is sensitive to positive control. The absence of activity on the medium has no effect.



Results of test bacteria with 16.8 mm zone of inhibition with a concentration of 0.03 mg/ml (Lay, 1994), while the dose can be said that bacteria test is sensitive to positive control. The absence of activity on the medium has no effect.

Figure 43. Results of antibiotic susceptibility test against *S. aureus* and *E. coli*

From the stability of the measured inhibition zone, in general the antibacterial properties of the supernatant produced by the symbiotic bacteria act as bactericidal against Gram positive bacteria and are merely bacteriostatic in Gram negative. Paper disc with a supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a 48 hour incubation period. While against Gram negative bacteria, around the disc paper shows the presence of inhibitory activity but gradually become turbid before the incubation period reaches 24 hours. Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Fernando B and Bruce RL Lay, 1994, 2020). Other factors that influence the ability of inhibitory inhibition are the concentration or intensity of antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of organic matter and the degree of acidity (pH) (Manisha DM and Shyamapada M, 2011; Sulistijowati and Mile, 2015).

Table 34. Results of measurement of inhibitory zone diameter of antibacterial compounds

Repetition	Diameter of zone inhibition (mm)					
	Gram positive			Gram negative		
	Symbiont bacterial (++)	Control (+)	Control (-)	Symbiont bacterial (++)	Control (+)	Control (-)
1	5,5	16	0	0	13,5	0
2	7,8	17,5	0	0	14	0
Average	6,7	16,8	0	0	13,8	0

The area of the symptomatic supernatant inhibition zone of *S. aureus* is 6.7 mm. According to Mounyr Balouiri et al., 2016; Edrada (1998) in Kusumadewi (2004) a measured inhibition zone of less than 10 mm belongs to a very weak and very active activity if and strong activity if the inhibition zone is greater than 15 mm. Testing of antibacterial activity of the symbiont bacteria supernatant obtained was still far from the results of the antibacterial activity of the tested compared to chloramphenicol control. This is because the antibacterial compound of the applied extracted symbiont bacteria is still was a supernatant with the containing secondary metabolites, it contains, but however, the test results have indicated

Commented [p22]: This is Discussion

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Commented [p23]: This is also discussion

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Space Before: 0 pt, Add space between paragraphs of the same style

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: English (Australia)

Formatted: Font: 9 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font color: Text 1

Formatted: Font color: Text 1

Formatted: Font color: Text 1

Formatted: Font: 9 pt

Formatted Table

Formatted: Font: 9 pt

Formatted: Font: 9 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

289 the presence of provide clear evidence of antibacterial activity. Generally the chemical structure of metabolites from marine
290 products is often different from the secondary differs from those of terrestrial origin. metabolite of land In fact, marine
291 bacteria are significant reservoirs of a plethora of bioactive molecules which have never been found in terrestrial organisms,
292 (Giovanna R., 2020 Gudbjarnason 1999 in Nofiani, 2005). -Seawater contains an active inhibitor agent for Gram positive
293 bacteria; according to (Garima K et al. Okami (1982)2) in in Nofiani (20052017) that seawater contains an active inhibitor
294 agent for organisms, seawater has the ability of inhibitors a gainst Gram positive bacteria.

295 ~~The activity of sea water inhibitor is not caused by faga or salinity but because there are antibacterial agents in~~
296 ~~seawater.~~

297 Based on the results of previous studies, most bacteria that live by associating with marine living creatures show great
298 potential in secondary metabolite secretion with antibacterial properties (Burgess et al., 1999; Armstrong et al., 2001;
299 Yanet et al., 2003 in Nofiani, 2005). Secondary metabolites are not used for growth and are formed from primary metabolites
300 under stress conditions. Examples of secondary metabolites are antibiotics, pigments, toxins, ecologic and symbiotic
301 competition effectors, pheromones, enzyme inhibitors, immunomodulating agents, antagonizing receptors and agonists,
302 pesticides, antitumor agents, and promoters of plant and animal growth (Nofiani, 2005).

303 Identification of Phenotype and Genotype of Symbiont Bacteria

304 ~~Known characteristics of the microscopic identification and biochemical tests of symbiont bacteria include the shape of a~~
305 ~~stem, non acidic, non spore forming, non motile, aerobically grown, negative catalase, and positive carbohydrates test.~~
306 ~~Based on the identification keys of Cowan and Steel (1993) referring to the 12th digit in the table of indications which~~
307 ~~indicates there are five types of bacteria suspected of having similar characters namely Brochothrix, Erysipelothrix,~~
308 ~~Lactobacillus, Arcanobacterium, and Arachmia.~~

310 Based on phenotypic identification results through cell staining and biochemical testing, symbiont bacteria has were
311 rod shaped, non acidic, non spore forming, non motile, aerobically grown, negative catalase, and positive to carbohydrate
312 tests. In general, the identification of microscopically selected isolates showed specific characteristics possessed by of
313 lactic acid bacteria (*Lactobacillus* spp.), such as round colonies, milky white, Gram positive with short stem cells, and
314 does without not form forming endospores (Desniar 2012 in Saskia, Davoodabadi et al. 20142015). The genus *Lactobacillus*
315 can be isolated from several different habitats, eg from milkfish intestine (Sulistijowati and Mile, 2015), bekasam products
316 (Ingratubun et al., 2013), up to coastal mangrove waters (Yahya et al., 2014).

317 ~~The DNA of the symbiont bacteria isolates was amplified using primers 9F and 1541R. The DNA~~
318 ~~bands used were relevant to the resulting PCR product of about 1400 base pairs. The sequence of DNA~~
319 ~~sequencing results of the 16S rDNA encoding of symbiont bacteria isolates showed an accurate score~~
320 ~~for species level with a similarity of ≥ 99% of the sequences present in GenBank. Then the species~~
321 ~~homology of the isolates tested was Lactobacillus plantarum. Classification of bacterial isolates are~~
322 ~~Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus~~
323 ~~plantarum.~~

```
Lactobacillus plantarum_100%
GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCAAGCAAACTCTGGTATTGATTGGTCTGCATCATGATTACAT
TTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCAGAAAGCGGGGATAACACCTGGAAACAGATGCTAATACCG
CATAACAACCTGGACCGCATGTCGGAGCTTGAAGATGGCTTCGGCTATCACTTTGGATGGTCCCGCGCGTATTAGCTAGATG
GTGGGTAACCGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCAAA
CTCCTACGGGAGGCGACAGTAGGGAATCTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCTGAGTGAAGAAGGGTTTC
GGCTCGTAAAACCTGTTGTTAAGAAGAACAATATCTGAGAGTAACTGTTCAAGTATTGACGGTATTAAACAGAAAGCCACGGCTA
ACTACGTGCCAGCAGCCGGTAATACGTAGGTGGCAAGCGTTGTCGGATTATTGGGCTAAAGCGGCAAGCGGCGGTTT
AAGTCTGATGTGAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAACTGGGAACTTGAAGTGCAGAAGAGGACAGTGAAGTC
CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAAGTACGCTGAGGCTC
GAAAGTATGGGTAGCAAAACAGGATTAGATACCCTGGTAGTCCATACCGTAACGATGAATGCTAAGTGTGGAGGGTTCCGCCCTC
TCAGTGTGTCAGCTAACGCATTAAAGCATTCCGCCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGCCCG
CACAAAGCGGTGGAGCATGTGGTTAATTCGAAGCTACGCGAAGAACCCTTACCAGGCTTGGACATACTATGCAAACTAAGAGATTA
GACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTGAGTCTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCTTATTATCAGTTGCCAGCATTAAAGTTGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGATG
ACGTCAAATCATATGCCCTTATGACCTGGGCTACACACGTGCTCAATGGATGGTACAACGATTCGCAACTCGCGAGAGTAAG
CTAATCTCTAAAGCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC
ATGCGCGGTGAATACGTTCCCGGCTTGTACACACCGCCGTCACACCATGAGAGTTTGAACACCCAAAAGTC
```

324 Figure 4. Sequens of 16S rDNA

- Formatted: Font: 10 pt
- Formatted: Font color: Text 1
- Formatted: Font color: Text 1
- Formatted: Font color: Text 1
- Formatted: Font: 10 pt
- Formatted: Font: 10 pt
- Commented [p24]: Moved to Introduction.
- Formatted: Font: 10 pt
- Formatted: Font: 10 pt
- Formatted: Indent: First line: 0 cm
- Commented [p25]: Not directly relevant
- Formatted: Font: 10 pt
- Formatted: Font: 10 pt, Italic
- Formatted: Font: 10 pt
- Formatted: Font: 10 pt
- Commented [p26]: Moved to Materials and Methods
- Commented [p27]: Moved to Results
- Formatted: Font: (Default) Arial, Bold
- Formatted: Left
- Formatted: Font: 9 pt
- Formatted: Font: 9 pt
- Formatted: Centered

392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427

Hudzieki. 2009. *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*. ASM Microbelibrary. American Society for Microbiology. http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol_7/04/2014

Ingratubun, J. A., Ijong, F. G., dan Onibala, H. 2013. *Isolasi dan Identifikasi Bakteri Asam Laktat pada Bakasang sebagai Starter Mikroba Produk Fermentasi*. Jurnal. Aquatic Science & Management, Edisi Khusus 1, 48-56. Pascasarjana, Universitas Sam Ratulangi.

Kalaivani, G., Hemalatha, N., dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria-Producing Antagonistic Bioactive Compounds Against Uti Pathogens*. International Journal of Pharma and Bio Sciences 2016 April; 7(2): (B) 395-405. India.

Kusumadewi, R. 2004. *Penapisan Awal Senyawa Bioaktif Antibakteri dari Melati Laut (Clerodendrum inermis)*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor: Bogor.

Lay, B. W. 1994. *Analisis Mikroba di Laboratorium*. PT. Raja Grafindo Persada: Jakarta

Lukman, J.B., Dwyana Z., Raya, I., Priocambodo, D. 2015. *Efektivitas Ekstrak Alga Eucheuma Cottonii, Turbinaria Decurrens, dan Ulva Reticulate Sebagai Antimikroba terhadap Streptococcus Mutans*. Jurnal. Jurusan Biologi FMIPA Universitas Hasanuddin: Makassar.

Nofiani, R. 2005. *Urgensi dan Mekanisme Biosintesis Metabolit Sekunder Mikroba Laut*. Jurnal Natur Indonesia 10(2), April 2008: 120-125.

O'Donnell K. 1993. *Fusarium and its near relatives*. In: Reynolds DR & Taylor JW (Eds) *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics* (pp.225-233). CAB International, Wallingford, UK.

Pitcher DG, Saunders NA, Owen RJ. 1989. *Rapid extraction of bacterial genomic DNA with guanidium thiocyanate*. Letters in Applied Microbiology 1989, 8: 151-156.

Pelezar, M.J dan Chan, E.C.S. 1986. *Dasar-dasar Mikrobiologi*. Diterjemahkan oleh Ratnasari, dkk. Edisi 1. UI Press, Jakarta

Sahara, F. N. I., Radjasa, O., K., dan Supriyantini, E. 2013. *Identifikasi Pigmen Karotenoid pada Bakteri Simbion Rumput Laut Kappahyeus alvarezii*. Journal Of Marine Research. Volume 2, Nomor 3, Tahun 2013, Halaman 58-67. Online di: <http://ejournal-slundip.ac.id/index.php/imr>.

Sartika, Ahmad, A., dan Natsir, H. 2014. *Potensi Antimikroba Protein Bioaktif dari Bakteri Simbion Alga Coklat Sargassum sp. Asal Perairan Pulau Laclea*. Jurnal: FMIPA Universitas Hasanuddin. Makassar.

Saskia, A. 2014. *Pengembangan Kultur Kering Bakteri Lactobacillus plantarum (SK5) asal Bekasam sebagai Kandidat Probiotik dengan Teknik Pengeringan Beku*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor: Bogor.

Siregar, A. F., Sabdono, A., dan Pringgienis, D. 2012. *Potensi Antibakteri Ekstrak Rumput Laut Terhadap Bakteri Penyakit Kulit Pseudomonas aeruginosa, Staphylococcus epidermidis, dan Micrococcus*. Journal Of Marine Research. Volume 1, Nomor 2, Tahun 2012, Halaman 152-160.

Sulistijowati, R. dan Mile, L. 2015. *Efektivitas Penghambatan Filtrat Asam Laktat Lactobacillus Sp. Hasil Isolasi Dari Usus Ikan Bandeng (Chanos chanos) Terhadap Bakteri Patogen*. Fakultas Perikanan dan Ilmu Kelautan Universitas Negeri Gorontalo.

Suparmi dan Sahri, A. 2009. *Kajian Pemanfaatan Sumber Daya Rumput Laut dari Aspek Industri Dan Kesehatan*. Jurnal: Sultan Agung Vol XLIV No. 96: 18. Fakultas Kedokteran Universitas Islam Sultan Agung.

White TJ, Bruns T, Lee S, and Taylor JW. 1990. *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. Pp. 315-322. In PCR Protocols: A guide to Methods and Applications. Academic Press, Inc. New York.

Yahya, Nursyam, H., Risjani, Y., dan Soemarno. 2014. *Karakteristik Bakteri di Perairan Mangrove Pesisir Kraton Pasuruan*. Jurnal. Ilmu Kelautan Maret 2014 Vol. 19(1):35-42. Pasa Sarjana Fakultas Pertanian, Universitas Brawijaya.

- Formatted: Font: 8 pt
- Formatted: Indent: Left: 0 cm, Hanging: 2 cm
- Formatted: Font: 8 pt
- Formatted: Justified, Indent: Left: 0 cm, Hanging: 2 cm
- Formatted: Indent: Left: 0 cm, Hanging: 2 cm
- Formatted: Font: 8 pt
- Formatted: Font: 8 pt, Not Bold
- Formatted: Indent: Left: 0 cm, Hanging: 2 cm, Line spacing: single
- Formatted: Indent: Left: 0 cm, Hanging: 2 cm
- Formatted: Font: 8 pt
- Formatted: Font: 8 pt, Not Bold
- Formatted: Indent: Left: 0 cm, Hanging: 2 cm, Line spacing: single
- Formatted: Font: 8 pt
- Formatted: Indent: Left: 0 cm, Hanging: 2 cm
- Formatted: Left: 1,8 cm, Right: 1,8 cm, Bottom: 2 cm

1 Antibacterial potential of symbiotic bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters

Formatted: jbd-dafpus8

2
3
4
5
6

7 **Abstract.** Brown seaweeds have the potential to produce bioactive compounds. It has been shown that the bacteria associated with
8 seaweeds are involved in the production of metabolites associated with their host. Microbes may be present as a living symbiotic in
9 association with other algae as epiphytes or endophytes. In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were
10 tested for antibacterial activity. A total of 14 isolates were isolated, 6 of which came from external tissue, while 8 isolates came
11 from internal tissue. Through the antagonistic test, 7 isolates showed inhibitory activity against *Staphylococcus aureus* and *Staphylococcus*
12 *aureus* and 1 isolate showed the inhibition against both *S.aureus* and *E.coli*. Phenotypic and genotypic identification showed that the
13 species-symbiotic bacteria species was *Lactobacillus plantarum*.

Formatted: Font: Not Bold, Italic

14 **Keywords:** bioassay, antagonistic, diffusion paper disc, *Lactobacillus plantarum*

15 INTRODUCTION

16 Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse, and unique
17 compounds (Bahare S et al. 2019). Many are the substances obtained from seaweed, such as alginates, carrageenan, and
18 agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea GZ et al. 2019). Other
19 compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory, and antifouling activity.
20 Therefore, seaweed can provide promising bioactive that can be used in the treatment of human diseases or new
21 antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. Much attention has
22 been paid to developing innovative projects for pharmaceuticals. Seaweed applications, especially in the design of new
23 antimicrobial drugs. Research for the identification of promising algal species, standardization of analytical methods,
24 isolation of compounds through integrated fractionation of bioassays, detailed chemical characterization and evaluation of
25 their safety, evaluation of synergistic effects between components, and efforts to improve yields. and lowering extraction
26 costs is needed (Marie JP et al. 2016).

27 It has been shown that the bacteria associated with seaweed as epiphytes or endophytes are involved within the
28 assembly of metabolites (Alessandro B et al. 2017). It's traditionally been used for children's fever, as a fertilizer,
29 repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011). *Turbinaria*
30 *conoides* belongs to the family of *Turbinaria*. The recent scientific trends target the pursuit for phytochemicals from marine algae due
31 to their numerous health-promoting effects, pathogens (Mark LW et al. 2016). Seaweeds can secrete secondary metabolites
32 with antibacterial properties (Emer S and Nissreen AG 2016). In the form of symbiotic mutualism. Algae provide needed
33 sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiotic bacteria isolates in
34 algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of infections acquired
35 from the community (Arumugama P et al. 2017) It here we evaluate the properties of the brown alga *Turbinaria conoides*
36 in producing bioactive compounds including the inhibition of human pathogens (Kalaivani et al. 2016). *T. conoides* is a
37 tropical marine algae widely distributed in coastal waters in Asia. We chose this algae following extensive trials on other
38 common macroalgae including *Sargassum* spp. and *Euchema cottonii*.

Formatted: Font: Italic

Formatted: Font: Not Bold

Formatted: Font: Not Bold

Formatted: Font: Not Bold

39 MATERIALS AND METHODS

40 Procedures

41 Sampling

42 Samples of *Turbinaria sp.* (about 1 kg wet weight) were taken from Lima island (S: -6.001051; E: 106.153804)
43 Samples were maintained in fresh seawater for laboratory analyses within 24 hours of collection.

44 Isolation of symbiotic bacteria producing antibacterial compounds

45 Epibionts were extracted from 15 grams of algae by rinsing with 30 mL of sterile sea water. The rinse water was
46 incubated in 30 mL of nutrient broth medium shaken at room temperature for 24 hours. Bioactive compound was

47 extracted by crushing 15 g of algawith a mortar and pestle with the addition of 15 ml of sterile seawater. The suspension
48 was incubated with 30 mL broth nutrient medium and shaken att room temperature for 24 hours.

49 After extraction process, the refreshed samples in the 30 ml broth nutrient medium were diluted into 9 ml broth nutrient
50 sterile 10^{-1} up to 10^{-5} . Each dilutions were grown on a plate count agar medium by incubate them at 37°C for 2 x 24 hours.
51 After incubating the petri dishes which were contain samples from each dilution, then the colonies bacteria from alga
52 would appear. The colonies bacteria producing antimicrobial compounds were characterized by a clear zone around the
53 colonies. Furthermore, the colonies with stable inhibition zones were collected by isolating them on slant agar medium,
54 with a clear code.

55 *Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria*

56 To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria, a qualitative test was
57 conducted directly by scratching round the isolates on the surface of the media that has been dispersed with test bacteria
58 (*Eschericia coli* and *Staphylococcus aureus*)^(ref). Media were incubated for 48 hours at 37°C . Each scratching round of
59 isolates was then marked by a unique code.

60 Inhibition zones were determined as those showing clear zones around the colony of symbiont bacteria isolates, for
61 both *Escheria coli* and *Staphylococcus aureus*. Strains that showed maximum antagonistic effect against tested pathogens
62 were identified. These were isolated and selected for further antibacterial testing by paper disc and identification of
63 phenotype and genotype.

64 *Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion*

65 Testing the supernatant of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was performed by the agar
66 diffusion method (Grela E et al. 2018)). The supernatant was obtained by separating the filtrate and supernatant by
67 centrifuge for 1 hour (25°C and 3000 rpm). Paper discs containing supernatant 40 μL and the negative control nutrient
68 broth 40 μL were left for 1 hour to reduce the water excess, and positive control chloramphenicol 0.01 mg/mL, were
69 placed on the surface of the Mueller Hinton Agar medium containing 1 mL test bacteria and incubated for 48 hours at 37°C .
70 The supernatant diffuses from the disc into the agar. If the organism is killed or inhibited by both the supernatant and
71 chloramphenicol as an antibiotic positive control, there will be no growth in the immediate area around the disc, this is
72 called the zone of inhibition. The zone sizes were compared to assess bioactivity as sensitive, resistant, or intermediate, in
73 each case the resistance zone where shows no colonies growth was measured by using a ruler to the nearest mm.

74 *Identification of phenotype and genotype of symbiont bacteria*

75 General bacterial identification (Phumudzo T, 2013) followed colony characteristic observations on liquid medium and
76 solid medium, observing cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and Biochemistry
77 test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase). The initial selection of isolates from mixed
78 cultures was carried out after enrichment and planting of *Turbinaria conoides* samples on the agar medium in pour plating.
79 Observation of medium incubated with temperature 37°C was done at incubation time reached 24 hours and 48 hours. The
80 data obtained from the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from
81 *Turbinaria conoides*. Determination of the type of bacteria was performed based on^{???}. Symbiont bacteria species were
82 determined by molecular testing.

83 The DNA of the symbiont bacteria isolated^{ds} was amplified using primers 9F and 1541R. The DNA bands used were
84 relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf
85 German) with a first pre-denaturation at 94°C for 90 seconds, followed by 30 cycles consisting of denaturation at 95°C
86 for 30 seconds, primary attachment at 50°C for 30 seconds, and extension at 72°C for 90 seconds. ~~After 30 eyeles~~
87 ~~completed,~~ followed by the elongation phase at 72°C for 5 min and cooling at 4°C for 20 min. Molecular
88 identification was done through partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES
89 method (Pitcher et al., 1989. Modified). PCR Amplification on 16S rDNA using Primer 9 F: 5' -- AAG GAG GTG ATC
90 CAG CC-3' and Primer 1541 R: 5' - GAG TTT GAT CCT GGC TCA G - 3' (White et al., 1990, O'Donnell, 1993). The
91 analysis of nitrogen base sequence readings was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic
92 Analyzer) (Applied Biosystems). The next sequenced raw data were trimmed and assembled using the BioEdit program
93 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequencing data were assembled in BLAST with genomic data
94 registered in DDBJ / DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>)

95 **RESULTS AND DISCUSSION**

96 **The Result of Symbiont Bacteria Isolation**

97 Samples consisted of the inside and outside of the algae, as many as 20 petri for 2 replications resulted in colonies with
98 the inhibit zone of 14 colonies, 6 of which were from epibionts, while the other 8 came from the algal tissue. The results
99 of identification of colonies grown on mixed cultures can be seen in Table 1. and identification of isolates isolated into
100 slant agar can be seen in Table 2.

Commented [A1]: Please incert a reference paper for this procedure???

Commented [A2]: Something is missing here

101 **Table 1.** Macroscopic forms of bacterial colonies

Colony code	Morphology of colonies			
	Shape	Color	Edges	Elevation
TUL ² -A1-2	Round	White	Flat	Convex shiny
TUL ² -A2-2	Round	White	Flat	Convex shiny
TUL ² -A3-2	Round	White	Flat	Convex shiny
TUL ² -A4-2	Round	White	Flat	Convex shiny
TUL ² -B1-2	Round	White	Crooked	Convex shiny
TUL ² -B2-2	Round	White	Crooked	Convex shiny
TUD ⁴ -C1-2	Round	White	Flat	Convex shiny
TUD ⁴ -C2-2	Round	White	Flat	Convex shiny
TUD ² -D1-2	Round	White	Crooked	Convex shiny
TUD ² -D2-2	Round	White	Crooked	Convex shiny
TUD ² -D3-2	Round	White	Crooked	Convex shiny
TUD ² -D4-2	Round	White	Crooked	Convex shiny
TUD ⁵ -E-2	Round	White	Flat	Convex shiny
TUD ³ -F-2	Round	White	Flat	Convex shiny

102 Information:

103 *The code of isolates TUL/TUD states the isolates originating from the outer/inner algae

104 ** The code of isolates (2), (4), (5), (3) states isolates obtained from the dilution

105 *** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according to the best inhibition zone of each colony observed on the plate

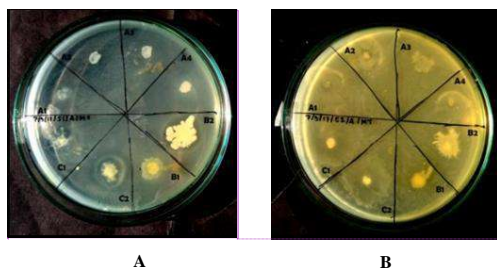
106 **** The code of number 2 identifies the isolate obtained from the second repeat

109 **Table 2.** Identification of the isolates on slant agar

Code of isolates	Solid medium	
	Shape	Color
TUL ² -A1-2	Spread	Milky white
TUL ² -A2-2	Spread	Milky white
TUL ² -A3-2	Spread	Milky white
TUL ² -A4-2	Spread	Milky white
TUL ² -B1-2	Rhizoidal	Cloudy white
TUL ² -B2-2	Rhizoidal	Cloudy white
TUD ⁴ -C1-2	Spread	Milky white
TUD ⁴ -C2-2	Spread	Milky white
TUD ² -D1-2	Rhizoidal	Cloudy white
TUD ² -D2-2	Rhizoidal	Cloudy white
TUD ² -D3-2	Rhizoidal	Cloudy white
TUD ² -D4-2	Rhizoidal	Cloudy white
TUD ⁵ -E-2	Spread	Milky white
TUD ³ -F-2	Spread	Milky white

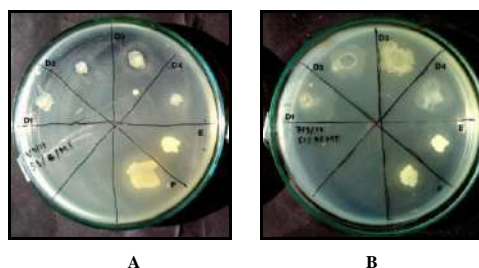
110 Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is
111 different for each species and it is characteristic of a particular species (Erin RS 2012).

112 **The Selection Results Symbiotic Bacteria Producing Antibacterial Compounds**



113 **Figure 1.** Symbiotic bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

Commented [A3]: The images are of poor quality, I would like to see in more detail these results, to determine real inhibition.

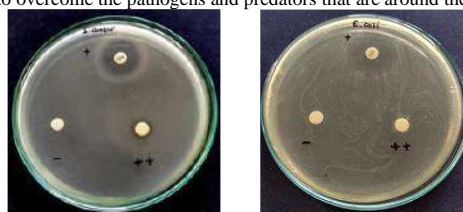


114 **Figure 2.** Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

115 Based on the results of the direct challenge test, 7 bacterial isolates from 14 isolates tested showed inhibitory activity
 116 against *S.aureus* and only 2 of the 7 isolates had inhibitory activity against *E.coli*. The isolate codes that have inhibitory
 117 zones against *S.aureus* bacteria are TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2, whereas TUD4-
 118 C1-2, And TUD4-C2-2 showed inhibition zones against both pathogenic bacteria being tested but the inhibitory activity
 119 against *E.coli* was not as good as its inhibition against *S.aureus*.

120 Isolates with showing inhibition were re-selected by looking at the best and largest clear zone. Isolates with code
 121 TUD4-C2-2 were isolates which had the best inhibition zone. Bacterial isolates derived from tissue showed better
 122 inhibition than isolates derived from epibionts. Inhibitory zone and diameter measurement results against *S.aureus* and
 123 *E.coli* can be seen in Figure 3 and Table 3. Positive controls have a broader spectrum in inhibiting both types of test
 124 bacteria with 16.8 mm inhibition against *S.aureus* and 13.8 mm against *E.coli*. Chloramphenicol with a concentration of
 125 0.03 mg on a paper disc is highly active if its inhibition zone is more than 18 mm (Mounyr B et al., 2016), while the dose
 126 of chloramphenicol (positive control) used is lower at 0.01 mg, so it can be said that bacteria Test is sensitive to positive
 127 control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone,
 128 so it can be ascertained that a supernatant still containing medium has no effect on the activity formed. From the stability
 129 of the measured inhibition zone, the antibacterial properties of the supernatant produced by the symbiotic bacteria act as
 130 inhibitors against Gram positive bacteria and are merely bacteriostatic for Gram negative bacteria. Paper disc with a
 131 supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a 48-hour incubation period.
 132 While against Gram negative bacteria, around the disc paper shows the presence of inhibitory activity but gradually
 133 become turbid before the incubation period reaches 24 hours.

134 The antibacterial compounds produced by symbiont bacteria isolates showed different inhibitory activity against both
 135 tested bacteria *S.aureus* and *E.coli*. According to Irma ESM (2011) the inner symbiotic bacteria generally have abundant
 136 populations and are specific microbes because they directly interact with the bioactive compounds produced from within
 137 the algae. While the symbiotic bacteria originating from the surface have a population that is less suspected because it
 138 requires higher defense power to overcome the pathogens and predators that are around the algae.



139 **Figure 3.** Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

140 Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Fernando
 141 B and Bruce RL, 2020). Other factors that influence the ability of inhibition are the concentration or intensity of
 142 antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of
 143 organic matter and the degree of acidity (pH) (Manisha DM and Shyamapada M 2011).

144 **Table 3.** Results of measurement of inhibitory zone diameter of antibacterial compounds

Repetition	Diameter of zone inhibition (mm)	
	Gram positive	Gram negative

Formatted: Font: Italic

	Symbiont bacterial (++)	Control (+)	Control (-)	Symbiont bacterial (++)	Control (+)	Control (-)
1	5,5	16	0	0	13,5	0
2	7,8	17,5	0	0	14	0
Average	6,7	16,8	0	0	13,8	0

145 The area of the symptomatic supernatant inhibition zone of *S. aureus* was 6.7 mm. According to Mounyr Balouiri et al.
146 2016, a measured inhibition zone of less than 10 mm shows weak activity and strong activity if the the inhibition zone is
147 greater than 15 mm. Testing of antibacterial activity of the symbiont bacteria supernatant obtained was still far from the
148 results of the antibiotic activity of the chloramphenicol control. This is because the antibacterial compound of the
149 extracted symbiont bacteria was a supernatant containing secondary metabolites. However, the test results provide clear
150 evidence of antibacterial activity. Generally the chemical structure of metabolites from marine products differs from those
151 of terrestrial origin. In fact, marine bacteria are significant reservoirs of a plethora of bioactive molecules which have
152 never been found in terrestrial organisms. (Giovanna R, 2020). Seawater contains an active inhibitor agent for Gram
153 positive bacteria (Garima K et al. 2017)

154 Identification of Phenotype and Genotype of Symbiont Bacteria

155 Based on phenotypic identification results through cell staining and biochemical tests, symbiont bacteria were rod
156 shaped, non acidic, non spore forming, non motile, aerobically grown, negative catalase, and positive to carbohydrate tests.
157 In general, the identification of selected isolates showed specific characteristics of lactic acid bacteria (*Lactobacillus*
158 spp.), such as round colonies, milky white, Gram positive with short stem cells, without forming endospores (Davoodabadi
159 et al. 2015).

Lactobacillus plantarum_100%

```
GCTCAGGACGAAACGCTGGCGGCGTGCCTAATACATGCAAGTCAAGCAAACTCTGGTATTGATTGGTGTTCATCATGATTTA
CATTTGAGTGAGTGGCGAACTGGTGAAGTAAACCGTGGGAAACCTGCCAGAAAGCGGGGGATAACACCTGGAAACAGATGCTAATA
CCGCATAAACAACCTGGACCGCATGGTCCGAGCTTGAAGATGGCTTCGGCTATCACTTTGGATGGTCCCGCGGGTATTAGCTAG
ATGGTGGGGTAAACCGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCC
AAACTCTACGGGAGGCAGCAGTAGGGAACTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCTGAGTGAAGAAGGGTT
TCGGCTCGTAAACTCTGTGTAAAGAAAGAACATATCTGAGAGTAACTGTTCAAGTATTGACGGTATTAAACGAGAAGCCACGGC
TAACTACGTGCCAGCAGCCGGTAAACGTAGGTGGCAAGCGTTGTCCGGATTATTGGCGTAAAGCGAGCGCAGGCGGTTTT
TTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAAGTGCATCCGAAACTGGGAAACTTGGTGCAGAAAGGACAGTGGAAAC
TCCATGTGTAGCGGTGAAATGCCGTAGATATATGGAAGAACACCAGTGGCGAAGCGCGCTGTCTGGTCTGTAACGACGCTGAGGC
TCGAAAGTATGGGTAGCAAAACAGGATTAGATACCGTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTGGAGGGTTCCGCC
CTTCAGTGTGACGCTAACGCATTAAGCATTCCCGCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
GCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCTACGCGAAGAACTTACCAGGCTTGACATACTGCAAATCTAAGAGATT
AGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGCTGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGGCCAACCTTATTATCAGTTCAGCAGCATTAAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAACCGGAGGAAGTGGGGAT
GACGTCAAATCATCATGCCCTTATGACCTGGGCTACACAGTGTCTACAATGGATGGTACAACGAGTTGCGAACTCCGCGAGAGTAA
GCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCTACATGAAGTGGGAATCGCTAGTAATCGCGGATCAG
CATGCCGCGGTGAATACGTTCCGGGCTTGTACACACCGCCCGTACACCATGAGAGTGTGTAACACCCAAAGTC
```

160
161 **Figure 4.** Sequens of 16S rDNA

162 Data for base sequence encoding gene of 16S rDNA shows that symbiont bacteria has accurate scores for species
163 levels with a similarity 100% of the sequences present in GenBank (Figure 4), The species homology of the tested isolate
164 was *Lactobacillus plantarum*.

165 In conclusion, *Turbinaria conoides* is commonly found in the gulf of Banten, Serang district, province of Banten. This
166 research showed that symbiont bacteria *Lactobacillus plantarum* are endophytic and potentially useful as an antibacterial
167 agent against common pathogens.

168 ACKNOWLEDGEMENTS

169 This paper and the research behind it would not have been possible without the exceptional support by Jakarta
170 Technical Fisheries University under the Applied Research Program of Fish Processing Technology Study Program. The
171 authors thank the Jakarta Technical Fisheries University for providing scientific publications fund.

REFERENCES

- 173 Alessandro B, Christine AM, Brendan FG. 2017. Marine macroalgae and their associated microbiomes as a source of antimicrobial
174 chemical diversity. *Eur J. of Phycol*, 52:4, 452-465
- 175 Andrea GZ, Miguel A. Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara. 2019. The potential of seaweeds as a source of
176 functional ingredients of prebiotic and antioxidant value. *Antioxid (Basel)*. 2019 Sep; 8(9): 406.
- 177 Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant and anticancer
178 properties of *Turbinaria conoides* (J. Agardh). *Clin Phytosci*. (2017) 3:5
- 179 Bahare S, Javad SR, Ana ML, Seca, Diana CGA, Pinto, Izabela M, Antonio T, Abhay PM, Manisha N, Wissam Z, Natália M, 2019.
180 Current trends on seaweeds: looking at chemical composition, phytopharmacology and cosmetic applications. *Mol*. 2019 Nov;
181 24(22): 4182.
- 182 Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015.
183 Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. *Pubmed*.
- 184 Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological
185 mechanisms and Applications. *Mar Drugs*. (2016) Apr; 14(4): 81.
- 186 Erin R sanders, 2012. Aseptic Laboratory Techniques: Plating Methods. *J Vis Exp*. 2012; (63): 3064.
- 187 Fernando Baquero and Bruce R. Levin , 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat Rev*.
188 *Microbiol*. (2020)
- 189 Garima Kapoor, Saurabh Saigal, and Ashok Elongavan, 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. *J*
190 *Anaesthesiol Clin Pharmacol*. 2017 Jul-Sep; 33(3): 300–305.
- 191 Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. res*
192 (2016)
- 193 Grela E., Kozłowska J., Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem.*;120(4):303–
194 311
- 195 Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol*.
196 2011;22:315–26.
- 197 Irma Esthela Soria-Mercado, Luis Jesús Villarreal-Gómez, Graciela Guerra Rivas and Nahara E. Ayala Sánchez, 2011. Bioactive
198 Compounds from Bacteria Associated to Marine in Algae. *Biotechnology: Molecular Studies and Novel Applications for Improved*
199 *Quality of Human L. BoD – Books on Demand*, 2012 (252)
- 200 Kalaivani , G., Hemalatha , N., dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria Producing*
201 *Antagonistic Bioactive Compounds Against Uti Pathogens*. *International Journal of Pharma and Bio Sci*. 2016 April; 7(2): (B) 395 –
202 405. India.
- 203 Manisha DM and Shyamapada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed*. 2011 Apr;
204 1(2): 154–160.
- 205 Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs*. 2016 Mar; 14(3): 52.
- 206 Mark LW, Philippe P, James SC, John AR, Sabeeha SM, Katherine EH, Alison GS, Mary EC, Susan HB, 2016. Algae as nutritional and
207 functional food sources: revisiting our understanding. *J. of Appl. Phycol*. volume 29 pages 949–982 (2017)
- 208 Mounyr B,*Moulay S and Saad KI, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal*. 2016 Apr;
209 6(2): 71–79.
- 210 Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for
211 Pharmacology. *Mar Drugs*. 2019 Aug; 17(8): 464.
- 212 Phumudzo T, Ronald N, Khayaletu N, Fhatuwani M, 2013. Bacterial species identification getting easier . *Afr J. of Biotechnol*. Vol.
213 12(41), pp. 5975-5982
- 214 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp.
215 315-322. In *PCR Protocols: A guide to Methods and Applications*, Academic Press, Inc., New York.

1 Antibacterial potential of symbiotic bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters

Formatted: jbd-dafpus8

2
3
4
5
6

7 **Abstract.** Brown seaweeds have the potential to produce bioactive compounds. It has been shown that the bacteria associated with
8 seaweeds are involved in the production of metabolites associated with their host. Microbes may be present as a living symbiotic in
9 association with other algae as epiphytes or endophytes. In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were
10 tested for antibacterial activity. A total of 14 isolates were isolated, 6 of which came from external tissue, while 8 isolates came
11 from internal tissue. Through the antagonistic test, 7 isolates showed inhibitory activity against *Staphylococcus aureus* and *Staphylococcus*
12 *aureus* and 1 isolate showed the inhibition against both *S.aureus* and *E.coli*. Phenotypic and genotypic identification showed that the
13 species-symbiotic bacteria species was *Lactobacillus plantarum*.

Formatted: Font: Not Bold, Italic

14 **Keywords:** bioassay, antagonistic, diffusion paper disc, *Lactobacillus plantarum*

15 INTRODUCTION

16 Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse, and unique
17 compounds (Bahare S et al. 2019). Many are the substances obtained from seaweed, such as alginates, carrageenan, and
18 agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea GZ et al. 2019). Other
19 compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory, and antifouling activity.
20 Therefore, seaweed can provide promising bioactive that can be used in the treatment of human diseases or new
21 antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. Much attention has
22 been paid to developing innovative projects for pharmaceuticals. Seaweed applications, especially in the design of new
23 antimicrobial drugs. Research for the identification of promising algal species, standardization of analytical methods,
24 isolation of compounds through integrated fractionation of bioassays, detailed chemical characterization and evaluation of
25 their safety, evaluation of synergistic effects between components, and efforts to improve yields. and lowering extraction
26 costs is needed (Marie JP et al. 2016).

27 It has been shown that the bacteria associated with seaweed as epiphytes or endophytes are involved within the
28 assembly of metabolites (Alessandro B et al. 2017). It's traditionally been used for children's fever, as a fertilizer,
29 repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011). *Turbinaria*
30 *conoides* belongs to the family of *Turbinaria*. The recent scientific trends target the pursuit for phytochemicals from marine algae due
31 to their numerous health-promoting effects, pathogens (Mark LW et al. 2016). Seaweeds can secrete secondary metabolites
32 with antibacterial properties (Emer S and Nissreen AG 2016). In the form of symbiotic mutualism. Algae provide needed
33 sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiotic bacteria isolates in
34 algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of infections acquired
35 from the community (Arumugama P et al. 2017) It here we evaluate the properties of the brown alga *Turbinaria conoides*
36 in producing bioactive compounds including the inhibition of human pathogens (Kalaivani et al. 2016). *T. conoides* is a
37 tropical marine algae widely distributed in coastal waters in Asia. We chose this algae following extensive trials on other
38 common macroalgae including *Sargassum* spp. and *Euchema cottonii*.

Formatted: Font: Italic

Formatted: Font: Not Bold

Formatted: Font: Not Bold

Formatted: Font: Not Bold

39 MATERIALS AND METHODS

40 Procedures

41 Sampling

42 Samples of *Turbinaria sp.* (about 1 kg wet weight) were taken from Lima island (S: -6.001051; E: 106.153804)
43 Samples were maintained in fresh seawater for laboratory analyses within 24 hours of collection.

44 Isolation of symbiotic bacteria producing antibacterial compounds

45 Epibionts were extracted from 15 grams of algae by rinsing with 30 mL of sterile sea water. The rinse water was
46 incubated in 30 mL of nutrient broth medium shaken at room temperature for 24 hours. Bioactive compound was

47 extracted by crushing 15 g of algawith a mortar and pestle with the addition of 15 ml of sterile seawater. The suspension
48 was incubated with 30 mL broth nutrient medium and shaken att room temperature for 24 hours.

49 After extraction process, the refreshed samples in the 30 ml broth nutrient medium were diluted into 9 ml broth nutrient
50 sterile 10^{-1} up to 10^{-5} . Each dilutions were grown on a plate count agar medium by incubate them at 37°C for 2 x 24 hours.
51 After incubating the petri dishes which were contain samples from each dilution, then the colonies bacteria from alga
52 would appear. The colonies bacteria producing antimicrobial compounds were characterized by a clear zone around the
53 colonies. Furthermore, the colonies with stable inhibition zones were collected by isolating them on slant agar medium,
54 with a clear code.

55 *Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria*

56 To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria, a qualitative test was
57 conducted directly by scratching round the isolates on the surface of the media that has been dispersed with test bacteria
58 (*Eschericia coli* and *Staphylococcus aureus*)^(ref). Media were incubated for 48 hours at 37°C . Each scratching round of
59 isolates was then marked by a unique code.

60 Inhibition zones were determined as those showing clear zones around the colony of symbiont bacteria isolates, for
61 both *Escheria coli* and *Staphylococcus aureus*. Strains that showed maximum antagonistic effect against tested pathogens
62 were identified. These were isolated and selected for further antibacterial testing by paper disc and identification of
63 phenotype and genotype.

64 *Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion*

65 Testing the supernatant of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was performed by the agar
66 diffusion method (Grela E et al. 2018)). The supernatant was obtained by separating the filtrate and supernatant by
67 centrifuge for 1 hour (25°C and 3000 rpm). Paper discs containing supernatant 40 μL and the negative control nutrient
68 broth 40 μL were left for 1 hour to reduce the water excess, and positive control chloramphenicol 0.01 mg/mL, were
69 placed on the surface of the Mueller Hinton Agar medium containing 1 mL test bacteria and incubated for 48 hours at 37°C .
70 The supernatant diffuses from the disc into the agar. If the organism is killed or inhibited by both the supernatant and
71 chloramphenicol as an antibiotic positive control, there will be no growth in the immediate area around the disc, this is
72 called the zone of inhibition. The zone sizes were compared to assess bioactivity as sensitive, resistant, or intermediate, in
73 each case the resistance zone where shows no colonies growth was measured by using a ruler to the nearest mm.

74 *Identification of phenotype and genotype of symbiont bacteria*

75 General bacterial identification (Phumudzo T, 2013) followed colony characteristic observations on liquid medium and
76 solid medium, observing cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and Biochemistry
77 test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase). The initial selection of isolates from mixed
78 cultures was carried out after enrichment and planting of *Turbinaria conoides* samples on the agar medium in pour plating.
79 Observation of medium incubated with temperature 37°C was done at incubation time reached 24 hours and 48 hours. The
80 data obtained from the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from
81 *Turbinaria conoides*. Determination of the type of bacteria was performed based on^{???}. Symbiont bacteria species were
82 determined by molecular testing.

83 The DNA of the symbiont bacteria isolated^{ds} was amplified using primers 9F and 1541R. The DNA bands used were
84 relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf
85 German) with a first pre-denaturation at 94°C for 90 seconds, followed by 30 cycles consisting of denaturation at 95°C
86 for 30 seconds, primary attachment at 50°C for 30 seconds, and extension at 72°C for 90 seconds. ~~After 30 eyeles~~
87 ~~completed,~~ followed by the elongation phase at 72°C for 5 min and cooling at 4°C for 20 min. Molecular
88 identification was done through partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES
89 method (Pitcher et al., 1989. Modified). PCR Amplification on 16S rDNA using Primer 9 F: 5' -- AAG GAG GTG ATC
90 CAG CC-3' and Primer 1541 R: 5' - GAG TTT GAT CCT GGC TCA G - 3' (White et al., 1990, O'Donnell, 1993). The
91 analysis of nitrogen base sequence readings was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic
92 Analyzer) (Applied Biosystems). The next sequenced raw data were trimmed and assembled using the BioEdit program
93 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequencing data were assembled in BLAST with genomic data
94 registered in DDBJ / DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>)

95 RESULTS AND DISCUSSION

96 The Result of Symbiont Bacteria Isolation

97 Samples consisted of the inside and outside of the algae, as many as 20 petri for 2 replications resulted in colonies with
98 the inhibit zone of 14 colonies, 6 of which were from epibionts, while the other 8 came from the algal tissue. The results
99 of identification of colonies grown on mixed cultures can be seen in Table 1. and identification of isolates isolated into
100 slant agar can be seen in Table 2.

Commented [A1]: Please incert a reference paper for this procedure???

Commented [A2]: Something is missing here

101 **Table 1.** Macroscopic forms of bacterial colonies

Colony code	Morphology of colonies			
	Shape	Color	Edges	Elevation
TUL ² -A1-2	Round	White	Flat	Convex shiny
TUL ² -A2-2	Round	White	Flat	Convex shiny
TUL ² -A3-2	Round	White	Flat	Convex shiny
TUL ² -A4-2	Round	White	Flat	Convex shiny
TUL ² -B1-2	Round	White	Crooked	Convex shiny
TUL ² -B2-2	Round	White	Crooked	Convex shiny
TUD ⁴ -C1-2	Round	White	Flat	Convex shiny
TUD ⁴ -C2-2	Round	White	Flat	Convex shiny
TUD ² -D1-2	Round	White	Crooked	Convex shiny
TUD ² -D2-2	Round	White	Crooked	Convex shiny
TUD ² -D3-2	Round	White	Crooked	Convex shiny
TUD ² -D4-2	Round	White	Crooked	Convex shiny
TUD ⁵ -E-2	Round	White	Flat	Convex shiny
TUD ³ -F-2	Round	White	Flat	Convex shiny

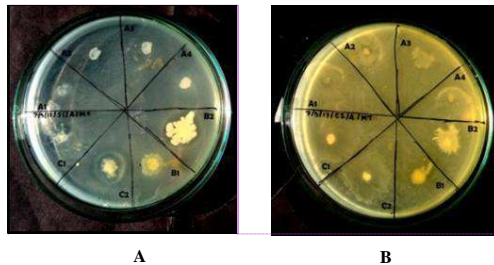
102 Information:
 103 *The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
 104 ** The code of isolates (2), (4), (5), (3) states isolates obtained from the dilution
 105 *** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the
 106 number code representing isolates in the order of the first, second, and so on according to the best inhibition zone of each colony observed
 107 on the plate
 108 **** The code of number 2 identifies the isolate obtained from the second repeat

109 **Table 2.** Identification of the isolates on slant agar

Code of isolates	Solid medium	
	Shape	Color
TUL ² -A1-2	Spread	Milky white
TUL ² -A2-2	Spread	Milky white
TUL ² -A3-2	Spread	Milky white
TUL ² -A4-2	Spread	Milky white
TUL ² -B1-2	Rhizoidal	Cloudy white
TUL ² -B2-2	Rhizoidal	Cloudy white
TUD ⁴ -C1-2	Spread	Milky white
TUD ⁴ -C2-2	Spread	Milky white
TUD ² -D1-2	Rhizoidal	Cloudy white
TUD ² -D2-2	Rhizoidal	Cloudy white
TUD ² -D3-2	Rhizoidal	Cloudy white
TUD ² -D4-2	Rhizoidal	Cloudy white
TUD ⁵ -E-2	Spread	Milky white
TUD ³ -F-2	Spread	Milky white

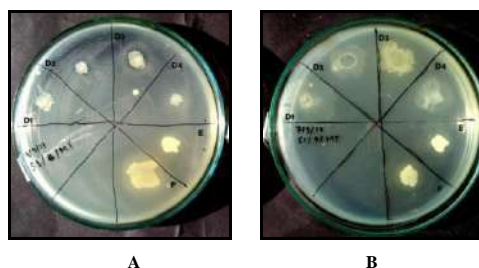
110 Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is
 111 different for each species and it is characteristic of a particular species (Erin RS 2012).

112 **The Selection Results Symbiotic Bacteria Producing Antibacterial Compounds**



113 **Figure 1.** Symbiotic bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

Commented [A3]: The images are of poor quality, I would like to see in more detail these results, to determine real inhibition.

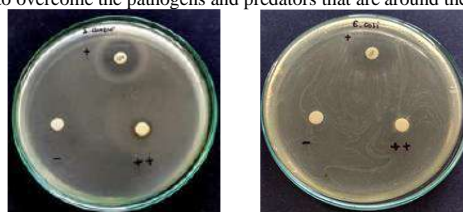


114 **Figure 2.** Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

115 Based on the results of the direct challenge test, 7 bacterial isolates from 14 isolates tested showed inhibitory activity
 116 against *S.aureus* and only 2 of the 7 isolates had inhibitory activity against *E.coli*. The isolate codes that have inhibitory
 117 zones against *S.aureus* bacteria are TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2, whereas TUD4-
 118 C1-2, And TUD4-C2-2 showed inhibition zones against both pathogenic bacteria being tested but the inhibitory activity
 119 against *E.coli* was not as good as its inhibition against *S.aureus*.

120 Isolates with showing inhibition were re-selected by looking at the best and largest clear zone. Isolates with code
 121 TUD4-C2-2 were isolates which had the best inhibition zone. Bacterial isolates derived from tissue showed better
 122 inhibition than isolates derived from epibionts. Inhibitory zone and diameter measurement results against *S.aureus* and
 123 *E.coli* can be seen in Figure 3 and Table 3. Positive controls have a broader spectrum in inhibiting both types of test
 124 bacteria with 16.8 mm inhibition against *S.aureus* and 13.8 mm against *E.coli*. Chloramphenicol with a concentration of
 125 0.03 mg on a paper disc is highly active if its inhibition zone is more than 18 mm (Mounyr B et al., 2016), while the dose
 126 of chloramphenicol (positive control) used is lower at 0.01 mg, so it can be said that bacteria Test is sensitive to positive
 127 control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone,
 128 so it can be ascertained that a supernatant still containing medium has no effect on the activity formed. From the stability
 129 of the measured inhibition zone, the antibacterial properties of the supernatant produced by the symbiotic bacteria act as
 130 inhibitors against Gram positive bacteria and are merely bacteriostatic for Gram negative bacteria. Paper disc with a
 131 supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a 48-hour incubation period.
 132 While against Gram negative bacteria, around the disc paper shows the presence of inhibitory activity but gradually
 133 become turbid before the incubation period reaches 24 hours.

134 The antibacterial compounds produced by symbiont bacteria isolates showed different inhibitory activity against both
 135 tested bacteria *S.aureus* and *E.coli*. According to Irma ESM (2011) the inner symbiotic bacteria generally have abundant
 136 populations and are specific microbes because they directly interact with the bioactive compounds produced from within
 137 the algae. While the symbiotic bacteria originating from the surface have a population that is less suspected because it
 138 requires higher defense power to overcome the pathogens and predators that are around the algae.



139 **Figure 3.** Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

140 Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Fernando
 141 B and Bruce RL, 2020). Other factors that influence the ability of inhibition are the concentration or intensity of
 142 antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of
 143 organic matter and the degree of acidity (pH) (Manisha DM and Shyamapada M 2011).

144 **Table 3.** Results of measurement of inhibitory zone diameter of antibacterial compounds

Repetition	Diameter of zone inhibition (mm)	
	Gram positive	Gram negative

Formatted: Font: Italic

	Symbiont bacterial (++)	Control (+)	Control (-)	Symbiont bacterial (++)	Control (+)	Control (-)
1	5,5	16	0	0	13,5	0
2	7,8	17,5	0	0	14	0
Average	6,7	16,8	0	0	13,8	0

145 The area of the symptomatic supernatant inhibition zone of *S. aureus* was 6.7 mm. According to Mounyr Balouiri et al.
146 2016, a measured inhibition zone of less than 10 mm shows weak activity and strong activity if the the inhibition zone is
147 greater than 15 mm. Testing of antibacterial activity of the symbiont bacteria supernatant obtained was still far from the
148 results of the antibiotic activity of the chloramphenicol control. This is because the antibacterial compound of the
149 extracted symbiont bacteria was a supernatant containing secondary metabolites. However, the test results provide clear
150 evidence of antibacterial activity. Generally the chemical structure of metabolites from marine products differs from those
151 of terrestrial origin. In fact, marine bacteria are significant reservoirs of a plethora of bioactive molecules which have
152 never been found in terrestrial organisms. (Giovanna R, 2020). Seawater contains an active inhibitor agent for Gram
153 positive bacteria (Garima K et al. 2017)

154 Identification of Phenotype and Genotype of Symbiont Bacteria

155 Based on phenotypic identification results through cell staining and biochemical tests, symbiont bacteria were rod
156 shaped, non acidic, non spore forming, non motile, aerobically grown, negative catalase, and positive to carbohydrate tests.
157 In general, the identification of selected isolates showed specific characteristics of lactic acid bacteria (*Lactobacillus*
158 spp.), such as round colonies, milky white, Gram positive with short stem cells, without forming endospores (Davoodabadi
159 et al. 2015).

Lactobacillus plantarum_100%

```
GCTCAGGACGAAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTA
CATTTGAGTGAGTGGCGAACTGGTGAAGTAAACCGTGGGAAACCTGCCAGAACCGGGGGATAACACCTGGAAACAGATGCTAATA
CCGCATAAACAACCTGGACCGCATGGTCCGAGCTTGAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGGTATTAGCTAG
ATGGTGGGGTAAACCGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACCGGCC
AAACTCCTACGGGAGGCGAGCAGTAGGGAACTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCTGAGTGAAGAAGGGTT
TCGGCTCGTAAACTCTGTGTAAAGAAAGAACATATCTGAGAGTAACTGTTCAAGTATTGACGGTATTAAACGAGAAGCCACGGC
TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTATTGGCGTAAAGCGAGCGCAGGCGGTTTT
TTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAAGTGCATCCGAAACTGGGAAACTTGGTGCAGAAAGGACAGCTGGAAC
TCCATGTGTAGCGGTGAAATGCCGTAGATATATGGAAGAACACCCAGTGGCGAAGCGCGCTGTCTGGTCTGTAACGACGCTGAGGC
TCGAAAGTATGGGTAGCAAAACAGGATTAGATACCGTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTGGAGGGTTCCGCC
CTTCAGTGTGCAAGTAAACGATTAAGCATTCCCGCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC
GCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCTACGCGAAGAACTTACCAGGCTTGACATACTGCAAATCTAAGAGATT
AGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGCTGTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGGCCAACCTTATTATCAGTTCAGCAGCATTAAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAACCGGAGGAAGTGGGGAT
GACGTCAAATCATCATGCCCTTATGACCTGGGCTACACAGTGTCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAA
GCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCTACATGAAGTGGGAATCGCTAGTAATCGCGGATCAG
CATGCCGCGGTGAATACGTTCCGGGCTTGTACACACCGCCGTCACACCATGAGAGTGTGTAACACCCAAAGTC
```

160
161 **Figure 4.** Sequens of 16S rDNA

162 Data for base sequence encoding gene of 16S rDNA shows that symbiont bacteria has accurate scores for species
163 levels with a similarity 100% of the sequences present in GenBank (Figure 4), The species homology of the tested isolate
164 was *Lactobacillus plantarum*.

165 In conclusion, *Turbinaria conoides* is commonly found in the gulf of Banten, Serang district, province of Banten. This
166 research showed that symbiont bacteria *Lactobacillus plantarum* are endophytic and potentially useful as an antibacterial
167 agent against common pathogens.

168 ACKNOWLEDGEMENTS

169 This paper and the research behind it would not have been possible without the exceptional support by Jakarta
170 Technical Fisheries University under the Applied Research Program of Fish Processing Technology Study Program. The
171 authors thank the Jakarta Technical Fisheries University for providing scientific publications fund.

REFERENCES

- 173 Alessandro B, Christine AM, Brendan FG. 2017. Marine macroalgae and their associated microbiomes as a source of antimicrobial
174 chemical diversity. *Eur J. of Phycol*, 52:4, 452-465
- 175 Andrea GZ, Miguel A. Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara. 2019. The potential of seaweeds as a source of
176 functional ingredients of prebiotic and antioxidant value. *Antioxid (Basel)*. 2019 Sep; 8(9): 406.
- 177 Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant and anticancer
178 properties of *Turbinaria conoides* (J. Agardh). *Clin Phytosci*. (2017) 3:5
- 179 Bahare S, Javad SR, Ana ML, Seca, Diana CGA, Pinto, Izabela M, Antonio T, Abhay PM, Manisha N, Wissam Z, Natália M, 2019.
180 Current trends on seaweeds: looking at chemical composition, phytopharmacology and cosmetic applications. *Mol*. 2019 Nov;
181 24(22): 4182.
- 182 Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015.
183 Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. *Pubmed*.
- 184 Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological
185 mechanisms and Applications. *Mar Drugs*. (2016) Apr; 14(4): 81.
- 186 Erin R sanders, 2012. Aseptic Laboratory Techniques: Plating Methods. *J Vis Exp*. 2012; (63): 3064.
- 187 Fernando Baquero and Bruce R. Levin , 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat Rev*.
188 *Microbiol*. (2020)
- 189 Garima Kapoor, Saurabh Saigal, and Ashok Elongavan, 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. *J*
190 *Anaesthesiol Clin Pharmacol*. 2017 Jul-Sep; 33(3): 300–305.
- 191 Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. res*
192 (2016)
- 193 Grela E., Kozłowska J., Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem.*;120(4):303–
194 311
- 195 Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol*.
196 2011;22:315–26.
- 197 Irma Esthela Soria-Mercado, Luis Jesús Villarreal-Gómez, Graciela Guerra Rivas and Nahara E. Ayala Sánchez, 2011. Bioactive
198 Compounds from Bacteria Associated to Marine in Algae. *Biotechnology: Molecular Studies and Novel Applications for Improved*
199 *Quality of Human L. BoD – Books on Demand*, 2012 (252)
- 200 Kalaivani , G., Hemalatha , N., dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria Producing*
201 *Antagonistic Bioactive Compounds Against Uti Pathogens*. *International Journal of Pharma and Bio Sci*. 2016 April; 7(2): (B) 395 –
202 405. India.
- 203 Manisha DM and Shyamapada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed*. 2011 Apr;
204 1(2): 154–160.
- 205 Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs*. 2016 Mar; 14(3): 52.
- 206 Mark LW, Philippe P, James SC, John AR, Sabeeha SM, Katherine EH, Alison GS, Mary EC, Susan HB, 2016. Algae as nutritional and
207 functional food sources: revisiting our understanding. *J. of Appl. Phycol*. volume 29 pages 949–982 (2017)
- 208 Mounyr B,*Moulay S and Saad KI, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal*. 2016 Apr;
209 6(2): 71–79.
- 210 Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for
211 Pharmacology. *Mar Drugs*. 2019 Aug; 17(8): 464.
- 212 Phumudzo T, Ronald N, Khayaletu N, Fhatuwani M, 2013. Bacterial species identification getting easier . *Afr J. of Biotechnol*. Vol.
213 12(41), pp. 5975-5982
- 214 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp.
215 315-322. In *PCR Protocols: A guide to Methods and Applications*, Academic Press, Inc., New York.

Antibacterial potential of symbiont bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters

Abstract. Brown seaweeds have the potential to produce bioactive compounds. ~~It has been shown that the bacteria~~ Bacteria associated with seaweeds are involved in the production of metabolites ~~associated with their host~~. Microbes may be present as a living symbiotic in association with other algae as epiphytes or endophytes. In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were tested for antibacterial activity. A total of 14 ~~isolates were found~~ bacteria were isolated, 6 of which ~~came 6~~ were isolated from external tissue, while 8 ~~came~~ from internal tissue. ~~Through the Results of~~ antagonistic test ~~revealed that~~, 7 isolates showed inhibitory activity against *Staphylococcus aureus* and only 1 isolate showed the inhibition against both *S.aureus* and *E.coli*. Phenotypic and genotypic ~~identification analysis~~ showed that the symbiont bacteria ~~species was~~ *Lactobacillus plantarum*.

Keywords: ~~bioassay~~ Bioassay, brown seaweed, antagonistic, diffusion paper disc, *Lactobacillus plantarum*

INTRODUCTION

Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse, and unique compounds (Bahare ~~S~~-et al. 2019). Many ~~are the~~ substances obtained from seaweed, such as alginates, carrageenan, and agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea ~~GZ~~-et al. 2019). Other compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory, and antifouling activity. Therefore, seaweed can provide promising bioactive that can be used in the treatment of human diseases or new antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. ~~Much attention has been paid to developing innovative projects for pharmaceuticals~~. Seaweed applications, ~~especially are particularly used~~ in the design of new antimicrobial drugs. Research for the identification of promising algal species, standardization of analytical methods, isolation of compounds through integrated fractionation of bioassays, detailed chemical characterization and evaluation of their safety, evaluation of synergistic effects between components, and efforts to improve yields, and lowering extraction costs is needed (Marie ~~JP~~-et al. 2016).

In later decades, ~~made~~ strides microbiological procedures have altogether made a difference in build-up phylogenetic affiliations of seaweed-related epi bacterial communities and endophytes. Be that as it may, there's inadequately prove that utilitarian connections for seaweed-bacterial intuitive can be built up and well caught on. Epiphytic bacterial communities are ~~quick rapid~~ colonizers of the ocean growth surface, some of the time versatile and able to quickly metabolize algal exudates (Singh ~~R.P~~ and Reddy ~~C.R.K~~, 2014). ~~It's~~ ~~It has~~ traditionally been used for children's fever, as a fertilizer, repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011). Seaweeds can secrete secondary metabolites with antibacterial properties (Emer ~~S~~ and Nissreen ~~AG~~ 2016). The ~~form of~~ symbiotic mutualism. Algae provide ~~needed essential~~ sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiont bacteria isolates ~~in as~~ algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of infections acquired from the community (Arumugama ~~P~~-et al. 2017). ~~*T. conoides* is a tropical marine alga widely distributed in coastal waters in Asia. It here we evaluate~~ This study evaluates the properties of the brown alga *Turbinaria conoides* in producing bioactive compounds including the inhibition of human pathogens (Kalaivani et al. 2016). ~~*T. conoides* is a tropical marine alga widely distributed in coastal waters in Asia. We chose this alga following extensive trials on other common macroalgae including *Sargassum* spp. and *Eucheuma cottonii*.~~

Commented [K1]: This is not the right way to write the references. Please correct it as according to the journal.

Commented [K2]: Incomplete line.

MATERIALS AND METHODS

Procedures

Sampling

Samples of *Turbinaria* sp. (about 1 kg wet weight) ~~were was~~ taken from Lima island (S: -6.001051; E: 106.153804). Samples were maintained in fresh seawater for laboratory ~~analyses analysis~~ within 24 hours of collection.

Formatted: Font: Not Italic

46 *Isolation of symbiont bacteria producing antibacterial compounds*

47 Epibionts were extracted from 15 grams of algae by rinse in with 30 mL of sterile seawater. The rinse water was
48 incubated in 30 mL of nutrient broth medium and shaken at room temperature for 24 hours. The bioactive compound was
49 extracted by crushing 15 g of alga with a mortar and pestle with the addition of 15 ml of sterile seawater. The suspension
50 was incubated with 30 mL nutrient broth nutrient medium and shaken at room temperature for 24 hours.

51 After the extraction process, the refreshed samples in the 30 ml broth nutrient medium were diluted into 9 ml broth
52 nutrient broth sterile-10⁻¹ up to 10⁻⁵. Each dilution was grown on a plate count agar medium by incubating them at 37 °C
53 for 2 x 24 hours. After incubating the petri dishes which contained samples from each dilution, then the colonies bacteria
54 from alga would appear. The colonies Colonies of bacteria producing that produce antimicrobial compounds were
55 characterized by a clear zone around the colonies. Furthermore, the colonies with stable inhibition zones were collected by
56 isolating them on a slant agar medium, with a clear code.

57 *Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria*

58 To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria For this, a qualitative test
59 was conducted carried out directly by scratching the isolates on the media that has been dispersed with two
60 test bacteria i.e. (*Escherichia coli* and *Staphylococcus aureus*), (Monte-J, et al 2014)). The media were was then incubated
61 for 48 hours at 37 °C. Each scratching round of isolates was then marked by a unique code.

62 Inhibition zones were determined as those showing clear zones around the colony of symbiont bacteria isolates, for
63 both *Escherichia coli* and *Staphylococcus aureus*. Strains that showed maximum antagonistic effect against tested
64 pathogens were identified. These were isolated and selected for further antibacterial testing by paper disc and identification
65 of phenotype and genotype. Strains showing maximum antagonistic effects were isolated and selected for antibacterial
66 testing by paper disc method. Further the strains were identified at the phenotypic and genotypic level.

67 *Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion*

68 Antibacterial Testing testing the supernatant of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus*
69 was performed by the agar diffusion method (Grela E et al. 2018)). The supernatant was obtained by separating the filtrate and
70 supernatant by was centrifuge for 1 hour (25 °C and 3000 rpm). Paper discs containing 40 µL supernatant was considered
71 as the treatment 40 µL and while 40 µL nutrient broth was used in the negative control nutrient broth 40 µL were left for 1
72 hour to reduce the water excess, and chloramphenicol (0.01 mg/mL) was used as positive control. chloramphenicol 0.01
73 mg/mL. After that the discs were placed on the surface of the Mueller Hinton Agar medium containing 1 mL test bacteria
74 and incubated for 48 hours at 37 °C. The supernatant diffuses from the disc into the agar. If the organism is killed or
75 inhibited by both the supernatant and chloramphenicol as an antibiotic positive control, there will be no growth in the
76 immediate area around the disc, this is called the zone of inhibition. The presence of a clear zone around the supernatant
77 and antibiotic discs was measured by the meter rule in mm. The zone sizes were compared to assess bioactivity as
78 sensitive, resistant, or intermediate, and the presence of a clear zone was measured by the meter rule in mm. in each case
79 the resistance zone where shows no colonies growth was measured by using a ruler to the nearest mm.

80 *Identification of phenotype and genotype of symbiont bacteria*

81 General bacterial identification was carried out on the basis of colony characteristic observations on liquid medium and
82 solid medium (Phumudzo T, 2013) followed colony characteristic observations on liquid medium and solid medium,
83 followed by observing cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and Biochemistry
84 biochemical test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase) as described by Phumudzo,
85 (2013). The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *Turbinaria*
86 *conoides* samples on the agar medium in pour plating. Observation of medium The plates were incubated with at 37 °C
87 temperature for 24 to 48 hours. 37 °C was done at incubation time reached 24 hours and 48 hours. The data obtained from
88 the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from *Turbinaria*
89 *conoides*. Determination of the type of bacteria was performed based on Phenotype and Genotype Symbiont bacteria species
90 were determined by molecular testing.

91 The DNA of the symbiont bacteria isolated was amplified using primers 9F and 1541R. The DNA bands used were
92 relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf
93 German) with a first pre-denaturation at 94 °C for 90 seconds, followed by 30 cycles consisting of denaturation at 95 °C
94 for 30 seconds, primary attachment at 50 °C for 30 seconds, and extension at 72 °C for 90 seconds. Followed followed
95 by the elongation phase at 72 °C for 5 min and cooling at 4 °C for 20 min. Molecular identification was done through
96 partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES method (Pitcher et al., 1989-
97 Modified). PCR Amplification on 16S rDNA using Primer 9 F: 5' - AAG GAG GTG ATC CAG CC-3' and Primer 1541
98 R: 5' - GAG TTT GAT CCT GGC TCA G - 3' (White et al., 1990, O'Donnell, 1993). The analysis of nitrogen base
99 sequence readings was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied
100 Biosystems). The next sequenced raw data were trimmed and assembled using the BioEdit program
101 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequencing data were assembled in BLAST with genomic data
102 registered in DDBJ / DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>)

Commented [K3]: I can't understand the meaning of this line. Why you incubate the extract of bioactive compounds in nutrient broth. Please check it.

Commented [K4]: Incomplete line. Rewrite it.

Commented [K5]: In the heading you have mentioned the paper disc and here you have written agar diffusion method.

Commented [K6]: This reference is not found in the reference section. Check it.

103

RESULTS AND DISCUSSION

104

The Result of Symbiont Bacteria Isolation

105

A total 14 colonies were isolated, of which 6 were from epibionts while the other 8 were from algal tissue. Samples consisted of the inside and outside of the algae, as many as 20 petri for 2 replications resulted in colonies with the inhibition zone of 14 colonies, 6 of which were from epibionts, while the other 8 came from the algal tissue. The results of the identification of colonies grown on mixed cultures can be seen in Table 1, and identification of isolates isolated into slant agar can be seen in Table 2. The macroscopic results of colonies on mixed cultures can be seen in Table 1, and on slant agar can be seen in Table 2.

107

108

109

110

111

Table 1. Macroscopic forms of bacterial colonies

Colony code	Morphology of colonies			
	Shape	Color	Edges	Elevation
TUL ² -A1-2	Round	White	Flat	Convex shiny
TUL ² -A2-2	Round	White	Flat	Convex shiny
TUL ² -A3-2	Round	White	Flat	Convex shiny
TUL ² -A4-2	Round	White	Flat	Convex shiny
TUL ² -B1-2	Round	White	Crooked	Convex shiny
TUL ² -B2-2	Round	White	Crooked	Convex shiny
TUD ⁴ -C1-2	Round	White	Flat	Convex shiny
TUD ⁴ -C2-2	Round	White	Flat	Convex shiny
TUD ² -D1-2	Round	White	Crooked	Convex shiny
TUD ² -D2-2	Round	White	Crooked	Convex shiny
TUD ² -D3-2	Round	White	Crooked	Convex shiny
TUD ² -D4-2	Round	White	Crooked	Convex shiny
TUD ⁵ -E-2	Round	White	Flat	Convex shiny
TUD ³ -F-2	Round	White	Flat	Convex shiny

112

Note:

113

*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae

114

** The code of isolates (2), (4), (5), (3) states isolates obtained from the dilution

115

*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according to the best inhibition zone of each colony observed on the plate

116

117

118

**** The code of number 2 identifies the isolate obtained from the second repeat

119

Table 2. Identification-Macroscopic form of the isolates on slant agar

Code of isolates	Solid medium	
	Shape	Color
TUL ² -A1-2	Spread	Milky white
TUL ² -A2-2	Spread	Milky white
TUL ² -A3-2	Spread	Milky white
TUL ² -A4-2	Spread	Milky white
TUL ² -B1-2	Rhizoidal	Cloudy white
TUL ² -B2-2	Rhizoidal	Cloudy white
TUD ⁴ -C1-2	Spread	Milky white
TUD ⁴ -C2-2	Spread	Milky white
TUD ² -D1-2	Rhizoidal	Cloudy white
TUD ² -D2-2	Rhizoidal	Cloudy white
TUD ² -D3-2	Rhizoidal	Cloudy white
TUD ² -D4-2	Rhizoidal	Cloudy white
TUD ⁵ -E-2	Spread	Milky white
TUD ³ -F-2	Spread	Milky white

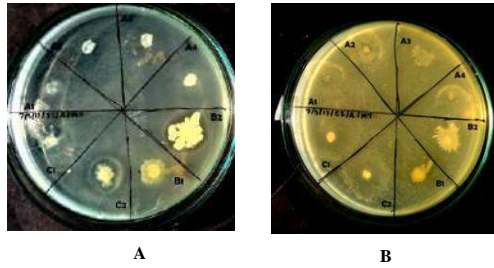
120

Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is different for each species and it is characteristic of a particular species (Erin RS 2012). Bacteria were isolated in a solid medium and the size of the colony was different for each species and was characteristic of a particular species (Erin 2012).

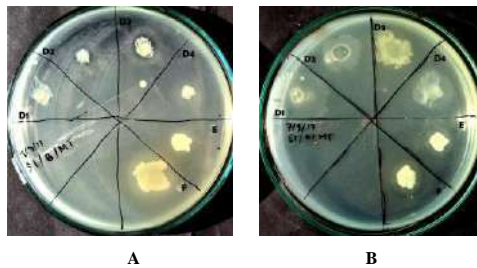
121

122

123 **The selection results symbiont bacteria producing antibacterial compounds**

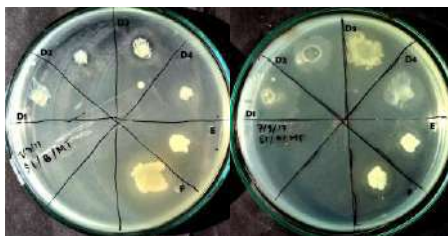


124 **Figure 1.** Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)



125 **Figure 2.** Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

126



127

128 **Figure 2.** Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

129 Based on the results of the direct challenge test, 7 bacterial isolates from 14 isolates tested showed inhibitory activity
130 against *S.aureus* and only 2 of the 7 isolates had inhibitory activity against *E.coli*. The isolate codes that have inhibitory
131 zones against *S.aureus* bacteria are TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2, whereas TUD4-
132 C1-2, and TUD4-C2-2 showed inhibition zones against both pathogenic bacteria being tested but the inhibitory activity
133 against *E.coli* was not as good as its inhibition against *S.aureus*. Based on the results of the direct challenge test, only 5
134 bacterial isolates i.e. TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2 showed inhibitory activity
135 against *S.aureus* whereas only 2 viz. TUD4-C1-2 and TUD4-C2-2 bacterial isolates showed inhibition zones against both
136 pathogenic bacteria. The inhibition activity was found to be lower in *E. coli* than in *S. aureus*.

137 Isolates with showing inhibition were re-selected by looking at the best and largest clear zone. Isolates with code
138 TUD4-C2-2 had the best inhibition zone. Bacterial isolates derived from algal tissue showed better inhibition than isolates
139 derived from epibionts. The inhibitory-inhibitory zone and diameter measurement results against *S.aureus* and *E.coli* can
140 be seen in Figure 3 and Table 3. Positive controls have a broader spectrum in inhibiting both types of test bacteria with
141 16.8 mm inhibition against *S.aureus* and 13.8 mm against *E.coli*. Positive controls showed 16.8 mm inhibition zone against
142 *S. aureus* and 13.8 mm inhibition zone against *E. coli*. Chloramphenicol with a concentration of 0.03 mg on a paper disc is
143 highly active if its inhibition zone is more than 18 mm (Mounyr B et al., 2016), while the dose of chloramphenicol

Formatted: Font: Italic

(positive control) used is was lower at less than 0.01 mg, so it can be said that bacteria Test-test is was found to be sensitive to the positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that a-supernatant still-containing-medium has no effect on the activity formed. From the stability of the measured inhibition zone, the The antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram-positive bacteria and are-were merely bacteriostatic for Gram-negative bacteria. Paper disc with a-supernatant applied to a Gram-positive bacterial plate indicates a stable clear zone even after a 48-hour incubation period. While against the Gram-negative bacteria, around the disc-paper shows the presence of inhibitory activity appeared around the disc paper, but it was gradually become turbid-turbulent before the incubation period reaches 24 hours.

The antibacterial compounds produced by symbiotic bacterial isolates showed different inhibitory activity against both tested bacteria *S.aureus* and *E.coli*. According to Irma ESM-et al. (2011), the inner symbiotic bacteria generally have abundant populations and are specific microbes because they directly interact with the bioactive compounds produced from within the algae. While the symbiotic bacteria originating from the surface have a population that is were less suspected-populated, because-as it requires-required higher defense power to overcome the pathogens and predators that are around the algae.

Commented [K7]: Discuss these results. Add references.



Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Fernando B and Bruce-RL, 2020). Other factors that influence-affect the ability-of inhibition-potential are the concentration or intensity of antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of organic matter, and the degree of acidity (pH) (Manisha DM and Shyamapada M 2011).

Table 3. Results of measurement of inhibitory zone diameter of antibacterial compounds

Repetition	The Diameter of zone inhibition (mm)					
	Gram-positive			Gram-negative		
	Symbiont bacterial (++)	Control (+)	Control (-)	Symbiont bacterial (++)	Control (+)	Control (-)
1	5.5	16	0	0	13.5	0
2	7.8	17.5	0	0	14	0
Average	6.7	16.8	0	0	13.8	0

The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Mounyr Balouiri et al. (2016): a-measured-less than 10 mm inhibition zone of less than 10 mm shows showed weak activity and strong activity if the inhibition zone is greater than 15 mm it indicates strong activity. Testing of antibacterial activity of the symbiont bacteria supernatant obtained was still far from the results of the antibiotic activity of the chloramphenicol control. This is because the antibacterial compound of the extracted symbiont bacteria was a the supernatant containing secondary metabolites. However, the test results provide clear evidence of antibacterial activity. Generally, the chemical structure of metabolites from marine products differs from those of terrestrial origin. Marine bacteria are significant reservoirs of a plethora of bioactive molecules that have never been found in terrestrial organisms: (Giovanna R, 2020). Seawater contains an active inhibitor agent for Gram-positive bacteria (Garima K et al. 2017)

Commented [K8]: Instead of Garima you should write Kapoor because author's surname is always written.

Identification of Phenotype and Genotype of Symbiont Bacteria

Based on the phenotypic observation comes about of phenotypic recognizable proof through cell recoloring and biochemical tests, the symbiont microscopic organisms were rod-shaped, non-acidic, non-spore-forming, non-motile, developing grow vigorously, catalase-negative, and a positive test for carbohydrates. In common, the distinguishing proof of chosen segregates appeared particular characteristics of lactic corrosive microscopic

Commented [K9]: Mentioned the name of the organism.

181 organisms (*Lactobacillus* spp.), Such as circular, smooth white, Gram-positive colonies with brief stem cells,
182 without shaping endospores (Davoodabadi et al. 2015).

183 The ~~Genotypic result through~~ molecular identification ~~is carried out~~ was done through partial genetic analysis of 16S
184 rDNA. PCR amplification results from the 16S region of bacterial ribosome DNA Nitrogen base sequences sorted from
185 symbiont bacterial isolates can be seen in figure 4. The sequencing information was ~~sequenced in impact with under the~~
186 ~~influence of~~ genomic information enlisted within the DDBJ / Japanese DNA Information Bank with 100%
187 strains grouping comes about. The characters of the antibacterial strains were indistinguishable from those of *Lactobacillus*
188 *plantarum*. Greatest Highest 100% personality, greatest score 2660, add up to score 2660, 100% inquiry scope, Esteem 0,
189 ~~was recorded to for~~ the taxon of adjacent microbes. [The classification of bacterial confines is as takes after: Microscopic
190 organisms; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus plantarum.]

192 Sequens of 16S rDNA

```
193 GCTCAGGACGAACCTGGCGGCTGCCTAATACATGCAAGTCGAACGAACCTGGTATTGATTGGTGCTTCATCATGAT
194 TTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCAGAAAGCGGGGATAACACCTGGAACAG
195 ATGCTAATACCGCATAACAACCTGGACCGCATGGTCCGAGCTTGAAGATGGCTTCGGCTATCACTTTGGATGGTCCCG
196 CGCGGTATTAGCTAGATGGTGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACA
197 TTGGGACTGAGACACGGCCAACTCCTACGGGAGGCAGCAGTAGGGAATCTCCACAATGGACGAAAGTCTGATGGAG
198 CAACGCCCGGTGAGTGAAGAAGGGTTTCGGCTCGTAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTC
199 GGTATTGACGGTATTTAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTG
200 TCCGGATTTATTGGCGTAAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTG
201 CATCGGAAACTGGGAACTTGAGTGCAGAAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGA
202 AGAACACCACTGGCGAAGGGCGTCTGGTCTGTAAGTACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAG
203 ATACCTGGTAGTCCATACCGTAAACGATGAATGTAAAGTGTGGAGGGTTCCCGCCTTCAGTGCTGCAGCTAACGCAT
204 TAAGCATTCGGCTGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCGCAAAAGCGGTGGAGC
205 ATGTGGTTAATTGCAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATTAAGAGATTAGACGTTCC
206 TTCGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTGATGATGTTGGGTTAAGTCCCGCAACGAGCG
207 CAACCCCTTATTATCAGTTGCCAGCATTAAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGA
208 TGACGTCAAATCATATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCCAACTCGCG
209 AGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCTACATGAAGTCGGAATCGCTAGT
210 AATCGCGGATCAGCATGCCGCGTGAATACGTTCCCGGGCCTGTACACACCGCCGTCACACCATGAGAGTTTGTAAACA
211 CCCAAAGTC
```

212 Figure 4. The arrangement of nitrogen bases sequenced from symbiont bacteria, A = adenine, T = thiamine, G = guanine, C = cytosine.

213
214 Data for base sequence encoding gene of 16S rDNA shows that symbiont bacteria have accurate scores for species
215 levels with a similarity 100% of the sequences present in GenBank. The species homology of the tested isolate was
216 *Lactobacillus plantarum*. *Lactobacillus plantarum* strains separated from dairy items appeared solid antimicrobial action
217 against the pointers strains of *Staphylococcus aureus*, *Salmonella* spp, and *Escherichia coli* (Hu ~~C-H~~, et al 2019).
218 The separation isolation of *L. plantarum* from Tibetan yaks was able to restrain the development of *E. coli* and *S. aureus*
219 (Wang ~~L~~, et al 2018). ~~Few Some Lactobacillus-lactobacillus~~ strains ~~appeared showed~~ antibacterial movement against
220 Enterobacteriaceae ~~which that~~ were safe ~~to for~~ carbapenems (CRE). This ~~impact effect~~ may have potential applications
221 through the utilize of the *Lactobacillus* strain as a starter culture in aged nourishments or as a nourishment additive to
222 control or avoid CRE contamination (Chen ~~C-C~~, et al 2019).

223 In conclusion, *Turbinaria conoides* ~~is was~~ commonly found in the gulf of Banten, Serang district, province of Banten.
224 This research ~~showed revealed~~ that symbiont bacteria *Lactobacillus plantarum* ~~are was~~ endophytic and potentially useful
225 as an antibacterial agent against common pathogens.

226 ACKNOWLEDGEMENTS

227 This ~~paper and the~~ The research behind this paper ~~it~~ would not have been possible without the exceptional support by
228 Jakarta Technical Fisheries University under the Applied Research Program of Fish Processing Technology Study
229 Program. The authors thank the Jakarta Technical Fisheries University for providing a scientific publications funding fund.

230 REFERENCES

231 Andrea GZ, Miguel A. Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara 2019. The potential of seaweeds as a source of
232 functional ingredients of prebiotic and antioxidant value. *Antioxid (Basel)*. 2019 Sep; 8(9): 406.
233 Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer
234 properties of *Turbinaria conoides* (J. Agardh). *Clin Phytosci*. 3:5

Commented [K10]: Please rewrite this line.

Formatted: Font: Italic

Commented [K11]: I can't interpret the meaning of this line. Please rewrite it

Formatted: Font: Italic

Formatted: Font: Italic

235 Bahare S, Javad SR, Ana ML, Seca, Diana CGA, Pinto, Izabela M, Antonio T, Abhay PM, Manisha N, Wissam Z, Natália M, 2019.
 236 Current trends on seaweeds: looking at chemical composition, phytopharmacology, and cosmetic applications. *Mol.* 2019 Nov;
 237 24(22): 4182.

238 Davoodabadi, Abolfazli; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015.
 239 Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Pubmed.
 240 Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological
 241 mechanisms and Applications. *Mar Drugs*. (2016) Apr; 14(4): 81.

242 Erin R sanders, 2012. Aseptic Laboratory Techniques: Plating Methods. *J Vis Exp*. 2012; (63): 3064.

243 Fernando Baquero and Bruce R. Levin, 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat Rev.*
 244 *Microbiol.* (2020)

245 Kapoor Garima Kapoor, Saigal Saurabh Saigal, and Elongavan Ashok Elongavan, 2017. Action and resistance mechanisms of
 246 antibiotics: A guide for clinicians. *J Anaesthesiol Clin Pharmacol*. 2017 Jul-Sep; 33(3): 300–305.

247 Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. res*
 248 (2016)

249 Grella E., Kozłowska J., Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem.*;120(4):303–
 250 311

251 Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol*.
 252 2011;22:315–26.

253 Hu, C. H., Ren, L. Q., Zhou, Y., & Ye, B. C. (2019). Characterization of antimicrobial activity of three *Lactobacillus plantarum* strains isolated from
 254 Chinese traditional dairy food. *Food science & nutrition*, 7(6), 1997–2005. <https://doi.org/10.1002/fsn3.1025>

255 Irma Esthela Soria-Mercado, Luis Jesús Villarreal-Gómez, Graciela Guerra Rivas and Nahara E. Ayala Sánchez, 2011. Bioactive
 256 Compounds from Bacteria Associated to Marine in Algae. *Biotechnology: Molecular Studies and Novel Applications for Improved*
 257 *Quality of Human L. BoD – Books on Demand*, 2012 (252)

258 Kalaivani, G., Hemalatha, N., dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria Producing*
 259 *Antagonistic Bioactive Compounds Against Uti Pathogens*. *International Journal of Pharma and Bio Sci*. 2016 April; 7(2): (B) 395 –
 260 405. India.

261 Chen C-C, Lai C-C, Huang H-L, Huang W-Y, Toh H-S, Weng T-C, Chuang Y-C, Lu Y-C, and Tang H-J (2019) Antimicrobial Activity
 262 of *Lactobacillus* Species Against Carbapenem-Resistant *Enterobacteriaceae*. *Front. Microbiol.* 10:789

263 Manisha DM and Shyamapada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed*. 2011 Apr;
 264 1(2): 154–160.

265 Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs*. 2016 Mar; 14(3): 52.

266 Monte, J., Abreu, A. C., Borges, A., Simões, L. C., & Simões, M. (2014). Antimicrobial Activity of Selected Phytochemicals against
 267 *Escherichia coli* and *Staphylococcus aureus* and Their Biofilms. *Pathogens (Basel, Switzerland)*, 3(2), 473–498.

268 Mounyr B,*Moulay S, and Saad KI, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal*. 2016 Apr;
 269 6(2): 71–79.

270 Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for
 271 Pharmacology. *Mar Drugs*. 2019 Aug; 17(8): 464.

272 Phumudzo T, Ronald N, Khayaletu N, Fhatuwani M, 2013. Bacterial species identification getting easier. *Afr J. of Biotechnol*. Vol.
 273 12(41), pp. 5975-5982

274 Singh R.P and Reddy C.R.K, 2014. Seaweed–microbial interactions: key functions of seaweed-associated bacteria. *FEMS Microbiol.*
 275 *Ecol*, Volume 88, Issue 2, April 2014, Pages 213–230.

276 Wang L, Zhang H, Rehman M.U, Khalid Mehmood K, Jiang X, Iqbal M, Tong X, Gao X, Li J,2018. Antibacterial activity of
 277 *Lactobacillus plantarum* isolated from Tibetan yaks. *J Microbial Pathogenesis*, Volume 115, Pages 293-298. ,

278 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp.
 279 315-322. In *PCR Protocols: A guide to Methods and Applications*, Academic Press, Inc., New York.

280

Commented [K12]: Write the full reference.

Commented [K13]: Check it again.

Commented [K14]: Write it in the correct way.

Commented [K15]: This reference is not found in the text. Check it.

Antibacterial potential of symbiont bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters

Abstract. Brown seaweeds have the potential to produce bioactive compounds. ~~It has been shown that the bacteria~~ Bacteria associated with seaweeds are involved in the production of metabolites ~~associated with their host~~. Microbes may be present as a living symbiotic in association with other algae as epiphytes or endophytes. In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were tested for antibacterial activity. A total ~~of~~ 14 isolates were found ~~bacteria were isolated~~, 6 of which ~~came~~ 6 were isolated from external tissue, while 8 ~~came~~ from internal tissue. ~~Through the Results of an~~ antagonistic test ~~revealed that~~, 7 isolates showed inhibitory activity against *Staphylococcus aureus* and ~~only~~ 1 isolate showed the inhibition against both *S.aureus* and *E.coli*. Phenotypic and genotypic ~~identification analysis~~ showed that the symbiont bacteria ~~species was~~ *Lactobacillus plantarum*.

Keywords: ~~bioassay~~ Bioassay, brown seaweed, antagonistic, diffusion paper disc, *Lactobacillus plantarum*

INTRODUCTION

Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse, and unique compounds (Bahare ~~S~~-et al. 2019). Many ~~are the~~ substances ~~are~~ obtained from seaweed, such as alginates, carrageenan, and agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea ~~GZ~~-et al. 2019). Other compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory, and antifouling activity. Therefore, seaweed can provide promising bioactive that can be used in the treatment of human diseases or new antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. ~~Much attention has been paid to developing innovative projects for pharmaceuticals~~. Seaweed applications, ~~especially are particularly used~~ in the design of new antimicrobial drugs. Research for the identification of promising algal species, standardization of analytical methods, isolation of compounds through integrated fractionation of bioassays, detailed chemical characterization and evaluation of their safety, evaluation of synergistic effects between components, and efforts to improve yields, and lowering extraction costs is needed (Marie ~~JP~~-et al. 2016).

In later decades, ~~made~~ strides microbiological procedures have altogether made a difference in build-up phylogenetic affiliations of seaweed-related epi bacterial communities and endophytes. Be that as it may, there's inadequately prove that utilitarian connections for seaweed-bacterial intuitive can be built up and well caught on. Epiphytic bacterial communities are ~~quick rapid~~ colonizers of the ocean growth surface, some of the time versatile and able to quickly metabolize algal exudates (Singh ~~R.P~~ and Reddy ~~C.R.K~~, 2014). ~~It's~~ ~~It has~~ traditionally been used for children's fever, as a fertilizer, repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011). Seaweeds can secrete secondary metabolites with antibacterial properties (Emer ~~S~~ and Nissreen ~~AG~~ 2016). The ~~form of~~ symbiotic mutualism ~~occurs as~~. Algae provide ~~needed essential~~ sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiont bacteria isolates ~~in as~~ algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of infections acquired from the community (Arumugama ~~P~~-et al. 2017). ~~T. conoides is a tropical marine alga widely distributed in coastal waters in Asia. Here we evaluate. This study evaluates the properties of the brown alga Turbinaria conoides in producing bioactive compounds including the inhibition of human pathogens (Kalaivani et al. 2016). T. conoides is a tropical marine alga widely distributed in coastal waters in Asia. We chose this alga following extensive trials on other common macroalgae including Sargassum spp. and Eucheuma cottonii.~~

Commented [K1]: This is not the right way to write the references. Please correct it as according to the journal.

Commented [K2]: Incomplete line.

Commented [ND3R2]: adjusted

MATERIALS AND METHODS

Procedures

Sampling

Samples of *Turbinaria* sp. (about 1 kg wet weight) ~~were were was~~ taken from Lima island (S: -6.001051; E: 106.153804) Samples were maintained in fresh seawater for laboratory ~~analyses analysis~~ within 24 hours of collection.

Formatted: Font: Not Italic

46 *Isolation of symbiont bacteria producing antibacterial compounds*

47 Epibionts were extracted from 15 grams of algae by rinse in with 30 mL of sterile seawater. The rinse water was
48 incubated in 30 mL of nutrient broth medium and shaken at room temperature for 24 hours. The bioactive compound was
49 extracted by crushing 15 g of alga with a mortar and pestle with the addition of 15 ml of sterile seawater. The suspension
50 was incubated with put into a 30 ml nutrient broth nutrient medium and shaken at room temperature for 24 hours.

51 After the extraction process, 1 ml of the refreshed samples from in the 30 ml broth nutrient was measured out and
52 homogenized in the sterile test tube containing 9 ml of medium were diluted in stages in sterile the 9 ml nutrient broth to
53 produce a 10⁻¹ dilution. This was done until 10⁻³ dilution is produced, for each dilute nutrient broth sterile 10⁻¹ up to 10⁻³.
54 Each dilution was grown on a plate count agar medium by incubating them at 37 °C for 2 x 24 hours. After incubating
55 the petri dishes which contained samples from each dilution, then the colonies bacteria from alga would appear. The
56 colonies Colonies of bacteria producing that produce antimicrobial compounds were characterized by a clear zone around
57 the colonies. Furthermore, the colonies with stable inhibition zones were collected by isolating them on a slant agar
58 medium, with a clear code.

59 *Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria*

60 To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria For this, a qualitative test
61 was conducted carried out directly by scratching the isolates on the surface of the media that has been dispersed with two
62 test bacteria i.e. (*Escherichia coli* and *Staphylococcus aureus*), (Monte-J, et al 2014). The media were was then incubated
63 for 48 hours at 37 °C. Each scratching round of isolates was then marked by a unique code.

64 Inhibition zones were determined as those showing clear zones around the colony of symbiont bacteria isolates; for
65 both *Escherichia coli* and *Staphylococcus aureus*. Strains that showed maximum antagonistic effect against tested
66 pathogens were identified. These were isolated and selected for further antibacterial testing by paper disc and identification
67 of phenotype and genotype. Strains showing maximum antagonistic effects were isolated and selected for antibacterial
68 testing by the paper disc diffusion method. Further, the strains were identified at the phenotypic and genotypic levels.

69 *Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion*

70 Antibacterial Testing testing the supernatant of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was
71 performed by the agar paper disc diffusion method (Grela E et al. 2018). The supernatant was obtained by separating the
72 filtrate and the supernatant by was centrifuged for 1 hour (25 °C and 3000 rpm). Paper discs containing 40 µL supernatant
73 was considered as the treatment 40 µL and while 40 µL nutrient broth was used in the negative control nutrient broth 40
74 µL were left for 1 hour to reduce the water excess, and chloramphenicol (0.01 mg/mL) was used as a positive control,
75 chloramphenicol 0.01 mg/mL. After that, the discs were placed on the surface of the Mueller Hinton Agar medium
76 containing 1 mL test bacteria and incubated for 48 hours at 37 °C. The supernatant diffuses from the disc into the agar. If
77 the organism is killed or inhibited by both the supernatant and chloramphenicol as an antibiotic positive control, there will
78 be no growth in the immediate area around the disc, this is called the zone of inhibition. The presence of a clear zone
79 around the supernatant and antibiotic discs was measured by the meter rule in mm. The zone sizes were compared to
80 assess bioactivity as sensitive, resistant, or intermediate, and the presence of a clear zone was measured by the meter rule
81 in mm. in each case the resistance zone where shows no colonies growth was measured by using a ruler to the nearest mm.

82 *Identification of phenotype and genotype of symbiont bacteria*

83 General bacterial identification was carried out based on on the basis of colony characteristic observations on liquid
84 medium and solid medium (Phumudzo T, 2013) followed colony characteristic observations on liquid medium and solid
85 medium, followed by observing cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and
86 Biochemistry biochemical test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase) as described by
87 Phumudzo, (2013). The initial selection of isolates from mixed cultures was carried out after enrichment and planting of
88 *Turbinaria conoides* samples on the agar medium in pour plating. Observation of medium The plates were incubated with
89 at 37°C temperature for 24 to 48 hours. 37°C was done at incubation time reached 24 hours and 48 hours. The data obtained
90 from the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from *Turbinaria*
91 *conoides*. Determination of the type of bacteria was performed based on Phenotype and Genotype Symbiont bacteria species
92 were determined by molecular testing.

93 The DNA of the symbiont bacteria isolated was amplified using primers 9F and 1541R. The DNA bands used were
94 relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf
95 German) with a first pre-denaturation at 94 °C for 90 seconds, followed by 30 cycles consisting of denaturation at 95 °C
96 for 30 seconds, primary attachment at 50 °C for 30 seconds, and extension at 72 °C for 90 seconds. Followed followed
97 by the elongation phase at 72 °C for 5 min and cooling at 4 °C for 20 min. Molecular identification was done through
98 partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES method (Pitcher et al., 1989:
99 Modified). PCR Amplification on 16S rDNA using Primer 9 F: 5' - AAG GAG GTG ATC CAG CC-3' and Primer 1541
100 R: 5' - GAG TTT GAT CCT GGC TCA G - 3' (White et al., 1990, O'Donnell, 1993). The analysis of nitrogen base
101 sequence readings was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied
102 Biosystems). The next sequenced raw data were trimmed and assembled using the BioEdit program

Commented [K4]: I can't understand the meaning of this line. Why you incubate the extract of bioactive compounds in nutrient broth. Please check it.

Commented [ND5R4]: Not incubated, but put into 30ml NB

Commented [K6]: Incomplete line. Rewrite it.

Commented [ND7R6]: corrected

Commented [K8]: Incomplete line. Rewrite it.

Commented [ND9R8]: corrected

Commented [K10]: In the heading you have mentioned the paper disc and here you have written agar diffusion method.

Commented [ND11R10]: corrected

Commented [K12]: This reference is not found in the reference section. Check it.

Commented [ND13R12]: adjusted

103 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequencing data were assembled in BLAST with genomic data
 104 registered in DDBJ / DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>)

105 **RESULTS AND DISCUSSION**

106 **The Result of Symbiont Bacteria Isolation**

107 A total of 14 colonies were isolated, of which 6 were from epibionts while the other 8 were from algal tissue. Samples
 108 consisted of the inside and outside of the algae, as many as 20 petri for 2 replications resulted in colonies with the inhibi
 109 zone of 14 colonies, 6 of which were from epibionts, while the other 8 came from the algal tissue. The results of the
 110 identification of colonies grown on mixed cultures can be seen in Table 1, and identification of isolates isolated into slant
 111 agar can be seen in Table 2. The macroscopic results of colonies on mixed cultures can be seen in Table 1, and on slant
 112 agar can be seen in Table 2.

113 **Table 1.** Macroscopic forms of bacterial colonies

Colony code	Morphology of colonies			
	Shape	Color	Edges	Elevation
TUL ² -A1-2	Round	White	Flat	Convex shiny
TUL ² -A2-2	Round	White	Flat	Convex shiny
TUL ² -A3-2	Round	White	Flat	Convex shiny
TUL ² -A4-2	Round	White	Flat	Convex shiny
TUL ² -B1-2	Round	White	Crooked	Convex shiny
TUL ² -B2-2	Round	White	Crooked	Convex shiny
TUD ⁴ -C1-2	Round	White	Flat	Convex shiny
TUD ⁴ -C2-2	Round	White	Flat	Convex shiny
TUD ² -D1-2	Round	White	Crooked	Convex shiny
TUD ² -D2-2	Round	White	Crooked	Convex shiny
TUD ² -D3-2	Round	White	Crooked	Convex shiny
TUD ² -D4-2	Round	White	Crooked	Convex shiny
TUD ⁵ -E-2	Round	White	Flat	Convex shiny
TUD ³ -F-2	Round	White	Flat	Convex shiny

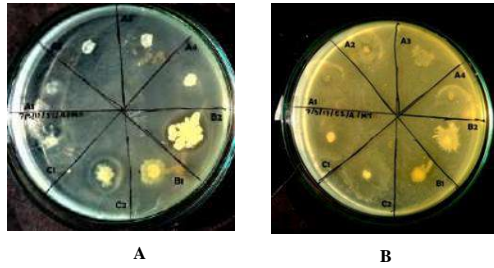
114 Note:
 115 *The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
 116 ** The code of isolates (2), (4), (5), (3) states isolates obtained from the dilution
 117 *** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the
 118 number code representing isolates in the order of the first, second, and so on according to the best inhibition zone of each colony observed
 119 on the plate
 120 **** The code of number 2 identifies the isolate obtained from the second repeat

121 **Table 2.** Identification-Macroscopic form of the isolates on slant agar

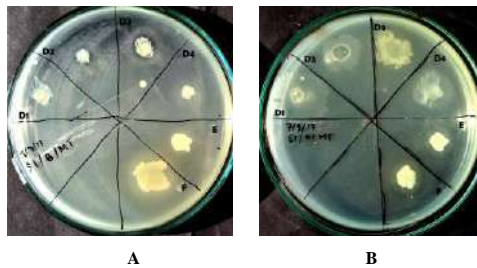
Code of isolates	Solid medium	
	Shape	Color
TUL ² -A1-2	Spread	Milky white
TUL ² -A2-2	Spread	Milky white
TUL ² -A3-2	Spread	Milky white
TUL ² -A4-2	Spread	Milky white
TUL ² -B1-2	Rhizoidal	Cloudy white
TUL ² -B2-2	Rhizoidal	Cloudy white
TUD ⁴ -C1-2	Spread	Milky white
TUD ⁴ -C2-2	Spread	Milky white
TUD ² -D1-2	Rhizoidal	Cloudy white
TUD ² -D2-2	Rhizoidal	Cloudy white
TUD ² -D3-2	Rhizoidal	Cloudy white
TUD ² -D4-2	Rhizoidal	Cloudy white
TUD ⁵ -E-2	Spread	Milky white
TUD ³ -F-2	Spread	Milky white

122 Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is
 123 different for each species and it is characteristic of a particular species (Erin RS 2012). Bacteria were isolated in a solid
 124 medium and the size of the colony was different for each species and was characteristic of a particular species (Erin 2012).

125 **The selection results symbiont bacteria producing antibacterial compounds**

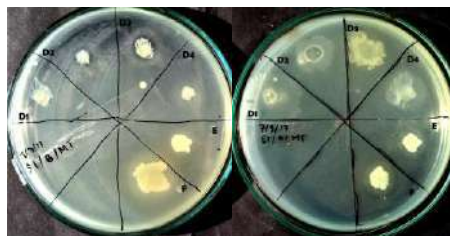


126 **Figure 1.** Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)



127 **Figure 2.** Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

128



129

130 **Figure 2.** Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

131 Based on the results of the direct challenge test, 7 bacterial isolates from 14 isolates tested showed inhibitory activity
132 against *S.aureus* and only 2 of the 7 isolates had inhibitory activity against *E.coli*. The isolate codes that have inhibitory
133 zones against *S.aureus* bacteria are TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2, whereas TUD4-
134 C1-2, and TUD4-C2-2 showed inhibition zones against both pathogenic bacteria being tested but the inhibitory activity
135 against *E.coli* was not as good as its inhibition against *S.aureus*. Based on the results of the direct challenge test, only 5
136 bacterial isolates i.e. TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2 showed inhibitory activity
137 against *S.aureus* whereas only 2 viz. TUD4-C1-2 and TUD4-C2-2 bacterial isolates showed inhibition zones against both
138 pathogenic bacteria. The inhibition activity was found to be lower in *E. coli* than in *S. aureus*.

139 Isolates with showing inhibition were re-selected by looking at the best and largest clear zone. Isolates with code
140 TUD4-C2-2 had the best inhibition zone. Bacterial isolates derived from algal tissue showed better inhibition than isolates
141 derived from epibionts. The inhibitory-inhibitory zone and diameter measurement results against *S.aureus* and *E.coli* can
142 be seen in Figure 3 and Table 3. Positive controls have a broader spectrum in inhibiting both types of test bacteria with
143 16.8 mm inhibition against *S.aureus* and 13.8 mm against *E.coli*. Positive controls showed 16.8 mm inhibition zone against
144 *S. aureus* and 13.8 mm inhibition zone against *E. coli*. Chloramphenicol with a concentration of 0.03 mg on a paper disc is
145 highly active if its inhibition zone is more than 18 mm (Mounyr B-et al., 2016), while the dose of chloramphenicol

Formatted: Font: Italic

(positive control) used is was lower at less than 0.01 mg, so it can be said that bacteria Test test is was found to be sensitive to the positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that a supernatant still containing medium has no effect on does not affect the activity formed. From the stability of the measured inhibition zone, the The antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram-positive bacteria and are were merely bacteriostatic for Gram-negative bacteria. As gram-positive symbiotic bacteria widely knows contain bacteriocins (Mezaini A et al. 2009 and Li D. Et al. 2015) bacteriocins from Gram-positive bacteria are generally not effective against Gram-negative bacteria (Smaoui et al. 2010). Paper disc with a supernatant applied to a Gram-positive bacterial plate indicates a stable clear zone even after a 48-hour incubation period. While against the Gram-negative bacteria, around the disc paper shows the presence of inhibitory activity appeared around the disc paper, but it was gradually become turbid turbulent before the incubation period reaches 24 hours.

The antibacterial compounds produced by symbiotic bacterial isolates showed different inhibitory activity against both tested bacteria *S.aureus* and *E.coli*. According to Irma ESM et al. (2011), the inner symbiotic bacteria generally have abundant populations and are specific microbes because they directly interact with the bioactive compounds produced from within the algae. While the symbiotic bacteria originating from the surface have a population that is were less suspected populated, because as it requires required higher defense power to overcome the pathogens and predators that are around the algae.



Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Fernando B and Bruce RL, 2020). Other factors that influence affect the ability of inhibition potential are the concentration or intensity of antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of organic matter, and the degree of acidity (pH) (Manisha DM and Shyamapada M 2011).

Table 3. Results of measurement of inhibitory zone diameter of antibacterial compounds

Repetition	The Diameter of zone inhibition (mm)					
	Gram-positive			Gram-negative		
	Symbiotic bacterial (++)	Control (+)	Control (-)	Symbiotic bacterial (++)	Control (+)	Control (-)
1	5.5	16	0	0	13.5	0
2	7.8	17.5	0	0	14	0
Average	6.7	16.8	0	0	13.8	0

The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Mounyr Balouiri et al. (2016), a measured less than 10 mm inhibition zone of less than 10 mm shows showed weak activity and strong activity if the inhibition zone is greater than 15 mm it indicates strong activity. Testing of antibacterial activity of the symbiotic bacteria supernatant obtained was still far from the results of the antibiotic activity of the chloramphenicol control. This is because of the antibacterial compound of the extracted symbiotic bacteria was a the supernatant containing secondary metabolites. However, the test results provide clear evidence of antibacterial activity. Generally, the chemical structure of metabolites from marine products differs from the those of terrestrial origin. Marine bacteria are significant reservoirs of a plethora of bioactive molecules that have never been found in terrestrial organisms. (Giovanna R, 2020). Seawater contains an active inhibitor agent for Gram-positive bacteria (Garima K Kapoor et al. 2017)

Identification of Phenotype and Genotype of Symbiotic Bacteria

Based on the phenotypic observation comes about of phenotypic recognizable proof through cell recoloring and biochemical tests, the The known characteristics of symbiotic bacteria through phenotypic observation by microscopic and biochemical tests — microscopic organisms were showing — rod-shaped, non-acidic, non-spore-forming, non-

Formatted: No underline

Commented [K14]: Discuss these results. Add references.

Commented [ND15R14]: adjusted

Commented [K16]: Instead of Garima you should write Kapoor because author's surname is always written.

Commented [ND17R16]: adjusted

Commented [K18]: Mentioned the name of the organism.

Commented [ND19R18]: adjusted

184 motile, developing ~~and grow vigorously~~ aerobically, negative catalase, and positive carbohydrate test, ~~catalase negative,~~
185 ~~and a positive test for carbohydrates.~~ In general, the selected isolate showed special characteristics possessed by lactic
186 ~~acid bacteria common, the distinguishing proof of chosen segregates appeared particular characteristics of~~
187 ~~lactic-corrosive-microscopic-organisms~~ (Lactobacillus spp.), ~~s~~ Such as circular, smooth white, Gram-positive colonies
188 with brief stem cells, without shaping endospores (Davoodabadi et al. 2015).

190 The Genotypic result through molecular identification ~~is carried out~~ was done through partial genetic analysis of 16S
191 rDNA. PCR amplification results from the 16S region of bacterial ribosome DNA Nitrogen base sequences sorted from
192 symbiont bacterial isolates can be seen in figure 4. The sequencing information was ~~sequenced in impact with under the~~
193 ~~influence of~~ genomic information enlisted within the DDBJ / Japanese DNA Information Bank with 100%
194 strains grouping comes about. The characters of the antibacterial strains were indistinguishable from those of *Lactobacillus*
195 *plantarum*. ~~Greatest~~ The highest 100% personality, greatest score 2660, add up to score 2660, 100% inquiry scope,
196 E esteem 0, was recorded ~~to for~~ the taxon of adjacent microbes. [The classification of ~~of the bacterial isolate is~~ Bacteria;
197 Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus plantarum.;

198 bacterial confines is as takes after: Microscopic organisms; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae;
199 Lactobacillus; Lactobacillus plantarum.]

201 Sequens of 16S rDNA

```
202 GCTCAGGACGAACCGTGGCGGCTGCCAATACATGCAAGTCGAACGAACCTCTGGTATTGATTGGTGCCTGCATCATGAT
203 TTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCAGAAGCGGGGATAACACCTGGAAACAG
204 ATGCATAATACGCATAACAACCTGGACCGCATGGTCCGAGCTTGAAGATGGCTTCGGCTATCATTGTTGGATGGTCCCG
205 CGCGTATTAGCTAGATGGTGGGGTAACGGCTACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACA
206 TTGGGACTGAGACACGGCCAAACTCTACGGGAGGCAGCAGTAGGGAATCTCCACAATGGACGAAAGTCTGATGGAG
207 CAACGCCCGTGAAGGAGGGTTTCGGCTCGTAAACTCTGTGTAAAGAAGAACAATCTGAGAGTAACGTGTTCA
208 GGTATTGACGGTATTAACAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTG
209 TCCGGATTTATGGGCGTAAAGCGAGCGCAGGCGTTTTTAAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTG
210 CATCGGAAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGAGCGGTGAAATGCGTAGATATATGGA
211 AGAACACCAAGTGGCGAAGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAG
212 ATACCCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTGGAGGGTTCCGCCCTTCAGTGTGCAGCTAACGCAT
213 TAAGCATTCCGCTGGGAGTACGGCCGAAGGCTGAAACTCAAAGGAATTGACGGGGCCCGCACAAAGCGTGGAGC
214 ATGTGGTTAATTGCAAGCTACCGGAAGAACCTTACCAGGCTTGACATACTATGCAAAATCTAAGAGATTAGACGTTCCC
215 TTCGGGACATGGATACAGGTGGTGCATGGTGTGTCGTCAGCTCGTGTGATGATGTTGGGTTAAGTCCCGCAACGAGCG
216 CAACCCITATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAACCGGAGGAAGGTGGGGA
217 TGACGTCAAATCATCATGCCCTTATGACCTGGGTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCG
218 AGAGTAAGCTAATCTCTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGT
219 AATCGCGATCAGCATCGCGCGTGAATACGTTCCCGGCTTGATCACACCCCGCTCACACCATGAGAGTTGTAAACA
220 CCCAAAGTC
```

221 Figure 4. The arrangement of nitrogen bases sequenced from symbiont bacteria, A = adenine, T = thiamine, G = guanine, C = cytosine.

223 Data for base sequence encoding gene of 16S rDNA shows that symbiont bacteria have accurate scores for species
224 levels with a similarity of 100% of the sequences present in GenBank. The species homology of the tested isolate was
225 *Lactobacillus plantarum*. *Lactobacillus plantarum* strains separated from dairy items appeared solid antimicrobial action
226 against the pointers strains of *Staphylococcus aureus*, *Salmonella spp.*, and *Escherichia coli* (Hu C.H., et al 2019).
227 The separation isolation of *L. plantarum* from Tibetan yaks was able to restrain the development of *E. coli* and *S. aureus*
228 (Wang L., et al 2018). Few Some ~~Lactobacillus~~ *Lactobacillus* strains appeared showed antibacterial movement against
229 Enterobacteriaceae which that were safe to for carbapenems (CRE). This impact effect may have potential applications
230 through the utilize of the *Lactobacillus* strain as a starter culture in aged nourishments or as a nourishment additive to
231 control or avoid CRE contamination (Chen C.C., et al 2019).

232 In conclusion, *Turbinaria conoides* was ~~is was~~ commonly found in the gulf of Banten, Serang district, province of
233 Banten. This research showed revealed that symbiont bacteria *Lactobacillus plantarum* are was endophytic and potentially
234 useful as an antibacterial agent against common pathogens.

235 ACKNOWLEDGEMENTS

236 This paper and the The research behind this paper it would not have been possible without the exceptional support by
237 Jakarta Technical Fisheries University under the Applied Research Program of Fish Processing Technology Study
238 Program. The authors thank the Jakarta Technical Fisheries University for providing a scientific publications funding fund.

Commented [K20]: Please rewrite this line.

Commented [ND21R20]: adjusted

Formatted: Font: Italic

Commented [K22]: I can't interpret the meaning of this line. Please rewrite it

Commented [ND23R22]: adjusted

Formatted: Indent: First line: 0 cm

Formatted: Font: Italic

Formatted: Font: Italic

REFERENCES

- 240 Andrea GZ, Miguel A. Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara; 2019. The potential of seaweeds as a source of
241 functional ingredients of prebiotic and antioxidant value. *Antioxid (Basel)*. 2019 Sep; 8(9): 406.
- 242 Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer
243 properties of *Turbinaria conoides* (J. Agardh). *Clin Phytosci*. 3:5
- 244 Bahare S, Javad SR, Ana ML, Seca, Diana CGA, Pinto, Izabela M, Antonio T, Abhay PM, Manisha N, Wissam Z, Natália M, 2019.
245 Current trends on seaweeds: looking at chemical composition, phytopharmacology, and cosmetic applications. *Mol*. 2019 Nov;
246 24(22): 4182.
- 247 [Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, Chuang YC, Lu YC, and Tang HJ. 2019. Antimicrobial Activity
248 of Lactobacillus Species Against Carbapenem-Resistant Enterobacteriaceae. *Front. Microbiol*. 10:789](#)
- 249 Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015.
250 Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria.
251 [PubMed Publish in Anaerobe, ISSN 1075-9964; Vol. 34; pp. 53 – 58.](#)
- 252 Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological
253 mechanisms and Applications. *Mar Drugs*. (2016) Apr; 14(4): 81.
- 254 Erin R sanders, 2012. Aseptic Laboratory Techniques: Plating Methods. *J Vis Exp*. 2012; (63): 3064.
- 255 Fernando Baquero and Bruce R. Levin, 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat Rev.*
256 *Microbiol*. (2020)
- 257 [Kapoor Garima Kapoor, Saigal Saurabh Saigal, and Elongavan Ashok Elongavan, 2017. Action and resistance mechanisms of
258 antibiotics: A guide for clinicians. *J Anaesthesiol Clin Pharmacol*. 2017 Jul-Sep; 33\(3\): 300–305.](#)
- 259 Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. res*
260 (2016)
- 261 Grela E., Kozłowska J., Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem.*;120(4):303–
262 311
- 263 Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol*.
264 2011;22:315–26.
- 265 [Hu, C. H., Ren, L. Q., Zhou, Y., & Ye, B. C. \(2019\). Characterization of antimicrobial activity of three Lactobacillus plantarum strains isolated from
266 Chinese traditional dairy food. *Food science & nutrition*, 7\(6\), 1997–2005. <https://doi.org/10.1002/fsn3.1025>](#)
- 267 [Irma Esthela-Soria-Mercado, Luis Jesús-Villarreal-Gómez LJV, Graciela-Guerra-Rivas GG, and Nahara E. Ayala-Sánchez NEA, 2011.
268 Bioactive Compounds from Bacteria Associated to Marine in Algae. *Biotechnology: Molecular Studies and Novel Applications for
269 Improved Quality of Human L. BoD – Books on Demand, 2012 \(25237\)*](#)
- 270 Kalaivani, G., Hemalatha, N., dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria Producing
271 Antagonistic Bioactive Compounds Against Uti Pathogens*. International Journal of Pharma and Bio Sci. 2016 April; 7(2): (B) 395 –
272 405. India.
- 273 [Kapoor Garima, Saigal Saurabh, and Elongavan Ashok, 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians.
274 *J Anaesthesiol Clin Pharmacol*. 2017 Jul-Sep; 33\(3\): 300–305.](#)
- 275 [Li D, Ni K, Pang H, Wang Y, Cai Y, Jin Q. 2015. Identification and Antimicrobial Activity Detection of Lactic Acid Bacteria Isolated
276 from Corn Stover Silage. *Asian-Australas J Anim Sci*. 2015 May; 28\(5\): 620–631.](#)
- 277 [Chen C-C, Lai C-C, Huang H-L, Huang W-Y, Toh H-S, Weng T-C, Chuang Y-C, Lu Y-C, and Tang H-J. \(2019\) Antimicrobial Activity
278 of Lactobacillus Species Against Carbapenem-Resistant Enterobacteriaceae. *Front. Microbiol*. 10:789](#)
- 279 Manisha DM and Shyamapada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed*. 2011 Apr;
280 1(2): 154–160.
- 281 Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs*. 2016 Mar; 14(3): 52.
- 282 [Mezaini A, Chihib N E, Bouras A D, Arroume N N, Hornez J P. 2009. Antibacterial Activity of Some Lactic Acid Bacteria Isolated
283 from an Algerian Dairy Product. *Journal of Environmental and Public Health*. Volume 2009.](#)
- 284 Monte, J., Abreu, A. C., Borges, A., Simões, L. C., & Simões, M. (2014). Antimicrobial Activity of Selected Phytochemicals against
285 *Escherichia coli* and *Staphylococcus aureus* and Their Biofilms. *Pathogens (Basel, Switzerland)*, 3(2), 473–498.
- 286 Mounyr B, Moulay S, and Saad KI, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal*. 2016 Apr;
287 6(2): 71–79.
- 288 [Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for
289 Pharmacology. *Mar Drugs*. 2019 Aug; 17\(8\): 464.](#) [O'Donnell, 1993. Fusarium and its Near Relatives. National Center for
290 Agriculture Utilization Research, USDA, ARS, 1815 N. University Street, Peoria, Illinois, 61604, USA.](#)
- 291 Phumudzo T, Ronald N, Khayaletu N, Fhatuwani M, 2013. Bacterial species identification getting easier. *Afr J. of Biotechnol*. Vol.
292 12(41), pp. 5975-5982
- 293 Singh R.P and Reddy C.R.K, 2014. Seaweed–microbial interactions: key functions of seaweed-associated bacteria. *FEMS Microbiol.*
294 *Ecol*. Volume 88, Issue 2, April 2014, Pages 213–230.
- 295 [Smaoui S, Elleuch L, Bejar W, Karray-Rebai I, Ayadi I, Jaouadi B, Mathieu F, Chouayekh H, Bejar S, Mellouli L. 2010. Inhibition of
296 fungi and gram-negative bacteria by bacteriocin BacTN635 produced by Lactobacillus plantarum sp. TN635. *Appl Biochem
297 Biotechnol*. 2010 Oct; 162\(4\):1132–46.](#)
- 298
- 299 Wang L, Zhang H, Rehman M.U, Khalid Mehmood K, Jiang X, Iqbal M, Tong X, Gao X, Li J, 2018. Antibacterial activity of
300 *Lactobacillus plantarum* isolated from Tibetan yaks. *J Microbial Pathogenesis*, Volume 115, Pages 293-298.
- 301
- 302
- 303 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp.
304 315-322. In *PCR Protocols: A guide to Methods and Applications*, Academic Press, Inc., New York.

Commented [K24]: Write it in the correct way.

Commented [ND25R24]: adjusted

Commented [K26]: Write the full reference.

Commented [ND27R26]: adjusted

Formatted: Font color: Auto

Commented [K28]: Check it again.

Commented [ND29R28]: adjusted

Commented [K30]: Write it in the correct way.

Formatted: Font: Not Bold

Formatted: Font: Not Bold

Formatted: No underline, Font color: Auto

Formatted: Font: Bold

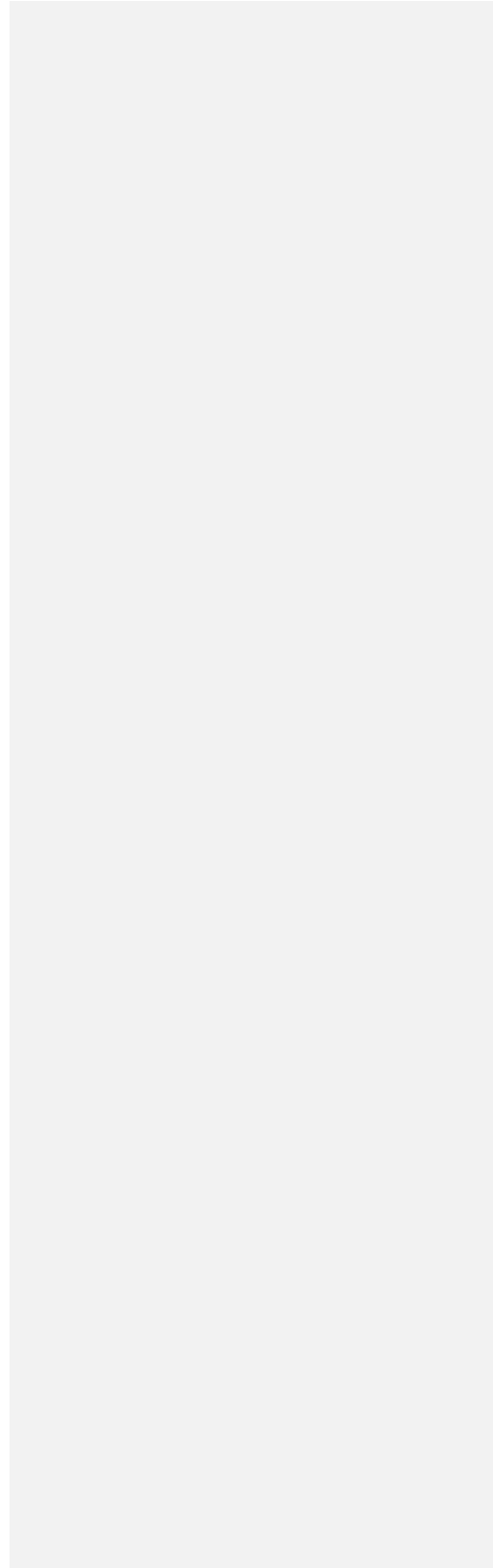
Commented [K31]: This reference is not found in the text. Check it.

Formatted: Font: Not Italic

Formatted: Indent: Left: 0 cm, Hanging: 0,5 cm

Formatted: Indent: Left: 0 cm, Hanging: 0,5 cm

305
306



Antibacterial potential of symbiont bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters

NIKEN DHARMAYANTI*, ARMA ANTI, RESMI RUMENTA SIREGAR, YULIATI H. SIPAHUTAR,
AEF PERMADI, ARPAN NASRI SIREGAR, RANDI BOKHI SALAMPESSY, SUJULIYANI,
SITI ZACHRO NURBANI, HENI BUDI PURNAMASARI

Program of Fish Processing Technology, Politeknik Ahli Usaha Perikanan. Jl. Pasar Minggu, South Jakarta 12520, Jakarta, Indonesia,
Tel.: +62-21-7806874, *email: niken.stp@gmail.com

Manuscript received: 7 October 2020. Revision accepted: 26 December 2020.

Abstract. Dharmayanti N, Anti A, Siregar RR, Sipahutar Y, Permadi A, Siregar AN, Salampessy RB, Sujuliyanti, Nurbani SZ, Purnamasari HB. 2021. Title. Biodiversitas 22: 373-377. Brown seaweeds have the potential to produce bioactive compounds. Bacteria associated with seaweeds are involved in the production of metabolites. Microbes may be present as a living symbiotic in association with other algae as epiphytes or endophytes. In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were tested for antibacterial activity. A total of 14 bacteria were isolated, of which 6 were isolated from external tissue, while 8 from internal tissue. Results of an antagonistic test revealed that 7 isolates showed inhibitory activity against *Staphylococcus aureus* and only 1 isolate showed the inhibition against both *S. aureus* and *Escherichia coli*. Phenotypic and genotypic analysis showed that the symbiont bacteria was *Lactobacillus plantarum*.

Keywords: Bioassay, brown seaweed, antagonistic, diffusion paper disc, *Lactobacillus plantarum*

INTRODUCTION

Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse, and unique compounds (Bahare et al. 2019). Many substances are obtained from seaweed, such as alginates, carrageenan, and agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea et al. 2019). Other compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory, and antifouling activity. Therefore, seaweed can provide promising bioactive that can be used in the treatment of human diseases or new antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. Seaweed applications are particularly used in the design of new antimicrobial drugs. Research for the identification of promising algal species, standardization of analytical methods, isolation of compounds through integrated fractionation of bioassays, detailed chemical characterization and evaluation of their safety, evaluation of synergistic effects between components, and efforts to improve yields and lowering extraction costs is needed (Marie et al. 2016).

In later decades, strides microbiological procedures have altogether made a difference in build-up phylogenetic affiliations of seaweed-related epi-bacterial communities and endophytes. Be that as it may, there is inadequately prove that utilitarian connections for seaweed-bacterial intuitive can be built up and well caught on. Epiphytic bacterial communities are rapid colonizers of the ocean growth surface, some of the time versatile and able to quickly metabolize algal exudates (Singh and Reddy 2014).

It has traditionally been used for children's fever, as a fertilizer, repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta and Abu-Ghannam 2011). Seaweeds can secrete secondary metabolites with antibacterial properties (Shannon and Abu-Ghannam 2016). The symbiotic mutualism occurs as algae provide essential sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiont bacteria isolates as algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of infections acquired from the community (Arumugama et al. 2017). *Turbinaria conoides* is a tropical marine alga widely distributed in coastal waters in Asia.

This study evaluates the properties of the brown alga *Turbinaria conoides* in producing bioactive compounds including the inhibition of human pathogens.

MATERIALS AND METHODS

Sampling

Samples of *Turbinaria conoides* (about 1 kg wet weight) were taken from Lima island, Serang City, Banten, Indonesia (S: -6.001051; E: 106.153804). Samples were maintained in fresh seawater for laboratory analysis within 24 hours of collection.

Isolation of symbiont bacteria producing antibacterial compounds

Bacteria were isolated in a solid medium and the size of the colony was different for each species and was characteristic of a particular species (Sanders 2012).

Epibionts were extracted from 15 grams of algae by rinse in with 30 mL of sterile seawater. The rinse water was incubated in 30 mL of nutrient broth medium and shaken at room temperature for 24 hours. The bioactive compound was extracted by crushing 15 g of alga with a mortar and pestle with the addition of 15 mL of sterile seawater. The suspension was insert into a 30 mL nutrient broth medium and shaken at room temperature for 24 hours.

After the extraction process, 1 mL of refresh samples were diluted in a 9 mL of sterile nutrient broth to make 10^{-1} dilution. This process was continued to achieve 10^{-5} dilution. Each dilution was grown on a plate count agar medium by incubating them at 37°C for 2 x 24 hours. Colonies of bacteria that produce antimicrobial compounds were characterized by a clear zone. Furthermore, the colonies with stable inhibition zones were collected by isolating them on a slant agar medium.

Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria

For this, a qualitative test was carried out directly by scratching the isolates on the surface of the media that has been dispersed with two test bacteria, i.e. *Escherichia coli* and *Staphylococcus aureus* (Monte et al. 2014)). The media was then incubated for 48 hours at 37°C. Each scratching round of isolates was then marked by a unique code. Inhibition zones were showing clear zones around the colony of symbiont bacteria isolates for both *E. coli* and *S. aureus*. Strains that showed maximum antagonistic effect against tested pathogens were identified. Strains showing maximum antagonistic effects were isolated and selected for antibacterial testing by the paper disc diffusion method. Further, the strains were identified at the phenotypic and genotypic levels.

Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion

Antibacterial testing of symbiont bacteria for inhibitory growth of *E. coli* and *S. aureus* was performed by the paper disc diffusion method (Grela et al. 2018). The supernatant was obtained by separating the filtrate and the supernatant was centrifuged for 1 hour (25°C and 3000 rpm). Paper discs containing 40 µL supernatant was considered as the treatment while 40 µL nutrient broth was used in negative control and chloramphenicol (0.01 mg/mL) was used as a positive control. After that, the discs were placed on the surface of the Mueller Hinton Agar medium containing 1 mL test bacteria and incubated for 48 hours at 37°C. The supernatant diffuses from the disc into the agar. The presence of a clear zone around the supernatant and antibiotic discs was measured by the meter rule in mm. The zone sizes were compared to assess bioactivity as sensitive, resistant, or intermediate, and the presence of a clear zone was measured by the meter rule in mm.

Identification of symbiont bacteria phenotype and genotype

General bacterial identification based on colony characteristic observations on liquid medium and solid medium followed by cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and biochemical test

(motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase) as described by Phumudzo et al. (2013). The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *T. conoides* samples on the agar medium. The plates were incubated at 37°C temperature for 24 to 48 hours. The data obtained from the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from *T. conoides*. The DNA of the symbiont bacteria isolated was amplified using primers 9F and 1541R. The DNA bands used were relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf German) with a first pre-denaturation at 94 ° C for 90 seconds, followed by 30 cycles consisting of denaturation at 95°C for 30 seconds, primary attachment at 50°C for 30 seconds, and extension at 72°C for 90 seconds, followed by the elongation phase at 72°C for 5 min and cooling at 4°C for 20 min. Molecular identification was done through partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES method (Pitcher et al. 1989 modified). PCR Amplification on 16S rDNA using Primer 9 F: 5'- AAG GAG GTG ATC CAG CC-3' and Primer 1541 R: 5' - GAG TTT GAT CCT GGC TCA G - 3' (White et al. 1990, O'Donnell 1993). The analysis of nitrogen base sequence readings was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data were trimmed and assembled using the BioEdit program (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequencing data were assembled in BLAST with genomic data registered in DDBJ/DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>).

RESULTS AND DISCUSSION

The result of symbiont bacteria isolation

A total of 14 colonies were isolated, of which 6 were from epibionts while the other 8 were from algal tissue. The macroscopic results of colonies on mixed cultures can be seen in Table 1, and on slant agar can be seen in Table 2.

The selection results symbiont bacteria producing antibacterial compounds

Based on the results of the direct challenge test, only 5 bacterial isolates i.e. TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2 showed inhibitory activity against *S.aureus* whereas only 2 viz. TUD4-C1-2 and TUD4-C2-2 bacterial isolates showed inhibition zones against both pathogenic bacteria. The inhibition activity was found to be lower in *E. coli* than in *S. aureus* (Figures 1 and 2).

Isolates with code TUD4-C2-2 had the best inhibition zone. Bacterial isolates derived from algal tissue showed better inhibition than isolates derived from epibionts. The inhibitory zone and diameter measurement results against *S. aureus* and *E. coli* can be seen in Figure 3 and Table 3.

Positive controls showed 16.8 mm inhibition zone against *S. aureus* and 13.8 mm inhibition zone against *E. coli*. Chloramphenicol with a concentration of 0.03 mg on a

paper disc is highly active if its inhibition zone is more than 18 mm (Mounyr et al. 2016), while the dose of chloramphenicol (positive control) used was less than 0.01 mg, so it can be said that bacteria test was found to be sensitive to the positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that supernatant does not affect the activity formed.

Table 1. Macroscopic forms of bacterial colonies.

Colony code	Morphology of colonies			
	Shape	Color	Edges	Elevation
TUL ² -A1-2	Round	White	Flat	Convex shiny
TUL ² -A2-2	Round	White	Flat	Convex shiny
TUL ² -A3-2	Round	White	Flat	Convex shiny
TUL ² -A4-2	Round	White	Flat	Convex shiny
TUL ² -B1-2	Round	White	Crooked	Convex shiny
TUL ² -B2-2	Round	White	Crooked	Convex shiny
TUD ⁴ -C1-2	Round	White	Flat	Convex shiny
TUD ⁴ -C2-2	Round	White	Flat	Convex shiny
TUD ² -D1-2	Round	White	Crooked	Convex shiny
TUD ² -D2-2	Round	White	Crooked	Convex shiny
TUD ² -D3-2	Round	White	Crooked	Convex shiny
TUD ² -D4-2	Round	White	Crooked	Convex shiny
TUD ⁵ -E-2	Round	White	Flat	Convex shiny
TUD ³ -F-2	Round	White	Flat	Convex shiny

Note: *The code of isolates TUL/TUD states the isolates originating from the outer/inner algae. ** The code of isolates (2), (4), (5), (3) states isolates obtained from the dilution. *** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according to the best inhibition zone of each colony observed on the plate. **** The code of number 2 identifies the isolate obtained from the second repeat

Table 2. Macroscopic form of the isolates on slant agar

Code of isolates	Solid medium	
	Shape	Color
TUL ² -A1-2	Spread	Milky white
TUL ² -A2-2	Spread	Milky white
TUL ² -A3-2	Spread	Milky white
TUL ² -A4-2	Spread	Milky white
TUL ² -B1-2	Rhizoidal	Cloudy white
TUL ² -B2-2	Rhizoidal	Cloudy white
TUD ⁴ -C1-2	Spread	Milky white
TUD ⁴ -C2-2	Spread	Milky white
TUD ² -D1-2	Rhizoidal	Cloudy white
TUD ² -D2-2	Rhizoidal	Cloudy white
TUD ² -D3-2	Rhizoidal	Cloudy white
TUD ² -D4-2	Rhizoidal	Cloudy white
TUD ⁵ -E-2	Spread	Milky white
TUD ³ -F-2	Spread	Milky white

Table 3. Results of inhibitory zone diameter

Repetition	The diameter of zone inhibition (mm)					
	Gram-positive			Gram-negative		
	Symbiotic bacterial (++)	Control (+)	Control (-)	Symbiotic bacterial (++)	Control (+)	Control (-)
1	5.5	16	0	0	13.5	0
2	7.8	17.5	0	0	14	0
Average	6.7	16.8	0	0	13.8	0

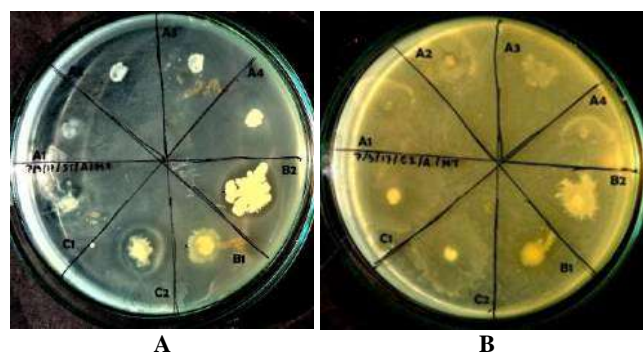


Figure 1. Symbiotic bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *Staphylococcus aureus* (A) and *Escherichia coli* (B)

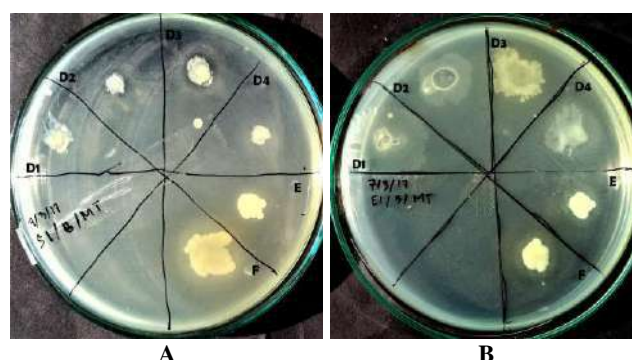


Figure 2. Symbiotic bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *Staphylococcus aureus* (A) and *Escherichia coli* (B)

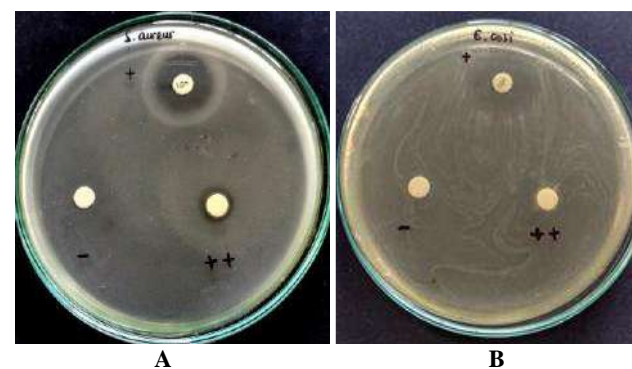


Figure 3. Results of antibiotic susceptibility test against: A. *Staphylococcus aureus* and B. *Escherichia coli*

The antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram-positive bacteria and were merely bacteriostatic for Gram-negative bacteria. As gram-positive symbiotic bacteria widely know contain bacteriocins (Mezaini et al. 2009; Li et al. 2015) bacteriocins from Gram-positive bacteria are generally not effective against Gram-negative bacteria (Smaoui et al. 2010). Paper disc with supernatant applied to a Gram-positive bacterial plate indicate a stable clear zone even after a 48-hour incubation period. While against the Gram-negative bacteria, the presence of inhibitory activity appeared around the disc paper, but it was gradually turbulent before the incubation period reaches 24 hours. The antibacterial compounds produced by symbiotic bacterial isolates showed different inhibitory activity against both tested bacteria *S.aureus* and *E.coli*. According to Soria-Mercado et al. (2011), the inner symbiotic bacteria generally have abundant populations and are specific because they directly interact with the bioactive compounds produced from within the algae. While the symbiotic bacteria were less populated, as it required higher defense power to overcome the pathogens and predators that are around the algae.

Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Baquero and Levin 2020). Other factors that affect the inhibition potential are the concentration or intensity of antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of organic matter, and the degree of acidity (pH) (Manisha and Shyamapada 2011).

The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Mounyr et al. (2016), less than 10 mm inhibition zone showed weak activity and if the inhibition zone is greater than 15 mm it indicates strong activity. Testing of antibacterial activity of the symbiotic bacteria supernatant obtained was still far from the results of the antibiotic activity of the

chloramphenicol control. This is because of the supernatant containing secondary metabolites. However, the test results provide clear evidence of antibacterial activity. Generally, the chemical structure of metabolites from marine products differs from the terrestrial origin. Marine bacteria are significant reservoirs of bioactive molecules that have never been found in terrestrial organisms (Barzkar et al. 2019). Seawater contains an active inhibitor agent for Gram-positive bacteria (Kapoor et al. 2017).

Identification of phenotype and genotype of symbiont bacteria

The known characteristics of symbiont bacteria through phenotypic observation and biochemical tests include rod-shaped, non-acidic, non-spore-forming, non-motile, grow aerobically, negative catalase, and positive carbohydrate test. In general, the selected isolate showed special characteristics possessed by lactic acid bacteria (*Lactobacillus* spp.), such as circular, smooth white, Gram-positive colonies with brief stem cells, without shaping endospores (Davoodabadi et al. 2015).

The molecular identification was done through partial genetic analysis of 16S rDNA. PCR amplification results from the 16S region of bacterial ribosome DNA Nitrogen base sequences sorted from symbiont bacterial isolates can be seen in Figure 4. The sequencing information was under the influence of genomic information enlisted within the DDBJ/Japanese DNA Information Bank with 100% strains grouping comes about. The characters of the antibacterial strains were indistinguishable from those of *Lactobacillus plantarum*. The highest 100% personality, greatest score 2660, add up to score 2660, 100% inquiry scope, E esteem 0, was recorded for the taxon of adjacent microbes. The classification of the bacterial isolate is Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; *Lactobacillus*; *Lactobacillus plantarum*.

Sequens of 16S rDNA

```
GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAG
TGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCAGAAAGCGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTT
GGACCGCATGGTCCGAGCTTGAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGCGTATTAGCTAGATGGTGGGGTAACGGCTCA
CCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTTGGACTGAGACACGGCCCAAACCTCTACGGGAGGCAGCAGTAGG
GAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCTGAGTGAAGAAGGGTTTCGGCTCGTAAAACCTCTGTTGTTAAAGAAGAA
CATATCTGAGAGTAACTGTTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTGTCCGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGC
ATCGGAACTGGGAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCCAGTGC
CGAAGGCGGCTGTCTGGTCTGTAACCTGACGCTGAGGCTCGAAAGTATGGGTAGCAAAACAGGATTAGATACCTGGTAGTCCATAACCGTAAA
CGATGAATGCTAAGTGTGGAGGGTTTCGGCCCTTCAGTGTGCAGCTAACGCATTAAGCATTCCGCTGGGGAGTACGGCCGCAAGGCTG
AAACTCAAAGGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTGAAAGCTACGCGAAGAACCTTACCAGGTCTTGACAT
ACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTGACGCTCGTGTGCGTGGATGTTGG
GTTAAGTCCCAGCAACGAGCGCAACCCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAAACCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACAGGTTGCGAACTCGCGAGA
GTAAGCTAATCTTTAAAGCCATTTCTCAGTTCGGATTGTAGGCTGCAACTCGCTACATGAAGTCGGAATCGCTAGTAAATCGCGGATCAGC
ATGCCGCGGTGAATACGTTCCCGGCTTGTACACACCCGCTCACACCATGAGAGTTTTGTAACACCCAAAGTC
```

Figure 4. The arrangement of nitrogen bases sequenced from symbiont bacteria, A: adenine, T: thiamine, G: guanine, C: cytosine

Data for base sequence encoding gene of 16S rDNA shows that symbiont bacteria have accurate scores for species levels with a similarity of 100% of the sequences present in GenBank. The species homology of the tested isolate was *L. plantarum*. *L. plantarum* strains separated from dairy items appeared solid antimicrobial action against the pointers strains of *S. aureus*, *Salmonella* spp, and *E. coli* (Hu et al. 2019). The isolation of *L. plantarum* from Tibetan yaks was able to restrain the development of *E. coli* and *S. aureus* (Wang et al. 2018). Some *Lactobacillus* strains showed antibacterial movement against Enterobacteriaceae that were safe for carbapenems (CRE). This effect may have potential applications through the utilize of the *Lactobacillus* strain as a starter culture in aged nourishments or as a nourishment additive to control or avoid CRE contamination (Chen et al. 2019).

In conclusion, *T. conoides* was commonly found in the gulf of Banten, Serang district, province of Banten. This research revealed that symbiont bacteria *L. plantarum* was endophytic and potentially useful as an antibacterial agent against common pathogens.

ACKNOWLEDGEMENTS

The research behind this paper would not have been possible without the exceptional support by Jakarta Technical Fisheries University, Indonesia under the Applied Research Program of Fish Processing Technology Study Program. The authors thank the Jakarta Technical Fisheries University for providing scientific publication funding.

REFERENCES

- Andrea GZ, Miguel A, Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara. 2019. The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. *Antioxid (Basel)* 8 (9): 406.
- Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer properties of *Turbinaria conoides* (J. Agardh). *Clin Phytosci* 3: 5. DOI: 10.1186/s40816-017-0042-y.
- Bahare S, Javad SR, Ana ML, Seca, Diana CGA, Pinto, Izabela M, Antonio T, Abhay PM, Manisha N, Wissam Z, Natália M. 2019. Current trends on seaweeds: looking at chemical composition, phytopharmacology, and cosmetic applications. *Molecules* 24 (22): 4182.
- Baquero F, Levin BR. 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat Rev Microbiol*. DOI: 10.1038/s41579-020-00443-1.
- Barzkar N, Jahromi ST, Poorsaheli HB, Vianello F. 2019. Metabolites from marine microorganisms, micro, and macroalgae: immense scope for pharmacology. *Mar Drugs* 17 (8): 464. DOI: 10.3390/md17080464.
- Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, Chuang YC, Lu YC, Tang HJ. 2019. Antimicrobial activity of *Lactobacillus* species against carbapenem-resistant Enterobacteriaceae. *Front Microbiol* 10: 789. DOI: 10.3389/fmicb.2019.00789.
- Davoodabadi A, Soltan Dallal MM, Rahimi Foroushani A, Douraghi M, Sharifi Yazdi MK, Amin Harati F. 2015. Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. *Anaerobe* 34: 53-58.
- Grela E, Kozłowska J, Grabowiecka. 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem* 120 (4): 303-311
- Gupta S, Abu-Ghannam N. 2011/Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol* 22: 315-326.
- Hu CH, Ren LQ, Zhou Y, Ye BC. 2019. Characterization of antimicrobial activity of three *Lactobacillus plantarum* strains isolated from Chinese traditional dairy food. *Food Sci Nutr* 7 (6): 1997-2005. DOI: 10.1002/fsn3.1025.
- Kapoor G, Saigal S, Elongavan A. 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. *J Anaesthesiol Clin Pharmacol* 33 (3): 300-305.
- Li D, Ni K, Pang H, Wang Y, Cai Y, Jin Q. 2015. Identification and antimicrobial activity detection of lactic acid bacteria isolated from corn stover silage. *Asian-Australas J Anim Sci* 28 (5): 620-631.
- Manisha DM, Shyamapada M. 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed* 1 (2): 154-160.
- Mari JP, Elena F, Herminia D. 2016. Antimicrobial action of compounds from marine seaweed. *Mar Drugs* 14 (3): 52. DOI: 10.3390/md14030052.
- Mezaini A, Chihib NE, Bouras AD, Arroume NN, Hornez JP. 2009. Antibacterial activity of some lactic acid bacteria isolated from an algerian dairy product. *J Environ Public Health* 2009: 678495. DOI: 10.1155/2009/678495..
- Monte J, Abreu AC, Borges A, Simões LC, Simões M. 2014. Antimicrobial activity of selected phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. *Pathogens (Basel, Switzerland)* 3 (2): 473-498.
- Mounyr B, Moulay S, Saad KI. 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal* 6 (2): 71-79.
- O'Donnell. 1993. *Fusarium* and its near relatives. In: Reynolds DR, Taylor JW (eds.). *The Fungal Holomorph Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematic*. CAB International, Wallingford, UK.
- Phumudzo T, Ronald N, Khayaletu N, Fhatuwani M. 2013. Bacterial species identification getting easier. *Afr J Biotechnol* 12 (41): 5975-5982.
- Sanders ER. 2012. Aseptic Laboratory Techniques: Plating Methods. *J Vis Exp* 63: e3064. DOI: 10.3791/3064.
- Shannon E, Abu-Ghannam N. 2016. Antibacterial derivatives of marine algae: An overview of pharmacological mechanisms and applications. *Mar Drugs* 14 (4): 81. DOI: 10.3390/md14040081.
- Singh RP, Reddy CRK. 2014. Seaweed-microbial interactions: key functions of seaweed-associated bacteria. *FEMS Microbiol Ecol* 88 (2): 213-230.
- Smaoui S, Elleuch L, Bejar W, Karray-Rebai I, Ayadi I, Jaouadi B, Mathieu F, Chouayekh H, Bejar S, Mellouli L. 2010. Inhibition of fungi and gram-negative bacteria by bacteriocin BacTN635 produced by *Lactobacillus plantarum* TN635. *Appl Biochem Biotechnol* 162 (4): 1132-46.
- Soria-Mercado IE, Villarreal-Gómez LJ, Guerra Rivas G, Ayala Sánchez NE. 2011. Bioactive compounds from bacteria associated to marine in algae. In: Sammour R (ed.) *Biotechnology: Molecular Studies and Novel Applications for Improved Quality of Human Life*, IntechOpen, UK. DOI: 10.5772/27842.
- Wang L, Zhang H, Rehman M.U, Khalid Mehmood K, Jiang X, Iqbal M, Tong X, Gao X, Li J. 2018. Antibacterial activity of *Lactobacillus plantarum* isolated from Tibetan yaks. *J Microbial Pathogenesis* 115: 293-298.
- White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A guide to Methods and Applications*, Academic Press, Inc., New York.

SURAT KLARIFIKASI JURNAL INTERNASIONAL SYARAT KHUSUS

Saya yang bertanda tangan dibawah ini

Nama : Niken Dharmayanti

Institusi : Politeknik AUP

Dengan ini melengkapi klarifikasi 3 File Jurnal Internasional syarat khusus penjelasan untuk File 3. Komunikasi dengan editor dan reviewer jurnal sesuai dengan yang diminta berbahasa Inggris dengan ini saya lengkapi.

Lampiran dari surat klarifikasi terdiri atas

File 1. Bukti *submission*

File 2. Bukti *accepted*

File 3. Komunikasi dengan *editor* dan *reviewer* jurnal

Semoga bisa memenuhi persyaratan jurnal khusus yang diminta.

Atas perhatiannya diucapkan terima kasih.

Hormat saya



Niken Dharmayanti

FILE 1. BUKTI SUBMISSION / TANGGAL 7 OKTOBER 2020

Dear **Editor-in-Chief**,

I herewith enclosed a research article,

Title:

Antibacterials Potential Symbiont Bacteria of Brown Algae (*Turbinaria Conoides*) Obtained from Indonesian waters

Author(s) name:

Niken Dharmayanti

Address

(Fill in your institution's name and address, your personal cellular phone and email)

Jakarta Fisheries Tehnical University, Pasar Minggu 12520, South Jakarta, Indonesia

Phone Number: 081385058734

Email: niken.stp@gmail.com

For possibility publication on the journal:

(fill in *Biodiversitas* or *Nusantara Bioscience* or mention the others)

Biodiversitas

Novelty:

Our research has identified antibacterial agents from endobionts associated with commonly-found brown seaweed in Indonesia. The anti-bacterial agents will have useful application in pharmaceuticals and other potential industrial application.

Statements:

This manuscript has not been published and is not under consideration for publication to any other journal or any other type of publication (including web hosting) either by me or any of my co-authors.

Author(s) has been read and agree to the Ethical Guidelines.

List of five potential reviewers

(Fill in names of five potential reviewers **that agree to review your manuscpt** and their **email** addresses. He/she should have Scopus ID and come from different institution with the authors; and from at least three different countries)

Place and date:

Jakarta, 07 October 2020

Sincerely yours,

(fill in your name, no need scanned autograph)

Niken Dharmayanti

Antibacterials Potential Symbiont Bacteria of Brown Algae (*Turbinaria conoides*) Obtained from Indonesian Waters

Niken Dharmayanti, Aef Permadi, Yuliati H Sipahutar, Resmi Rumenta Siregar, Arpan Nasri Siregar, Randi Bokhi Salampessy, Sujuliyani, Arma Anti

Study program of Fish Processing Technology, Jakarta Fisheries Technical University, Pasar Minggu 12520, South Jakarta, Indonesia

ABSTRACT

Brown seaweed has the potential to produce bioactive compounds. It has been shown that the bacteria associated with seaweed are involved in the production of metabolites associated with their host. Microbes may be present as a living symbiotic in association with other algae as epiphytes or endophytes. In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were tested for antibacterial activity. A total of 14 isolates were isolated, 6 of which came from external tissue, while 8 isolates came from internal tissue. Through the antagonistic test, 7 isolates showed inhibitory activity against *Staphylococcus aureus* and 1 isolate showed the inhibition against both *S.aureus* and *E.coli*. Phenotypic and genotypic identification showed that the species symbiont bacteria was *Lactobacillus plantarum*.

Keywords: bioassay, antagonistic, diffusion paper disc, *Lactobacillus plantarum*.

INTRODUCTION

Indonesia is the largest producer of seaweed in the world (FAO 2016) cultured in nearshore coastal regions. Seaweeds produce diverse bioactive metabolites with antibacterial, antiviral, antifungal and cytotoxic properties (Zainuddin and Malina, 2009 in Siregar et al., 2012). It has been shown that the bacteria associated with seaweed as epiphytes or endophytes are involved in the production of metabolites (Sartika et al, 2014, Kalaivani et al., 2016). Symbiont bacteria isolates in algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of the form of symbiotic mutualism. Algae provide needed sites and nutrients, while the bacteria encourage growth and protect the algal surface against pathogens (Hollants et al., 2012 in Sartika et al, 2014). Seaweeds can secrete secondary metabolites with antibacterial properties (Burgess et al., 1999; Armstrong et al., 2001; Yanet et al., 2003 in Nofiani, 2005).

Here we evaluate the properties of the brown alga *Turbinaria conoides* in producing bioactive compounds including the inhibition of human pathogens (Kalaivani et al., 2016). *T. conoides* is a tropical marine alga widely distributed in coastal waters in Asia. We chose this alga following extensive trials on other common macroalgae including *Sargassum* spp. and *Euchema cottonii*.

MATERIALS AND METHODS

Procedures

Sampling

Samples of *Turbinaria sp.* (about 1 kg wet weight) were taken from Lima island (S: -6.001051; E: 106.153804) Samples were maintained in fresh seawater for laboratory analyses within 24 hour of collection.

Isolation of Symbiont Bacteria Producing Antibacterial Compounds

Epibionts were extracted from 15 grams of algae by rinsing with 30 mL of sterile sea water. The rinse water was incubated in 30 mL of nutrient broth medium shaken at room temperature for 24 hours. Bioactive compound were extracted by crushing 15 g of algae with a mortar and pestle with the addition of 15 ml of sterile seawater. The suspension was incubated with 30 mL broth nutrient medium and shaken at room temperature for 24 hours.

After extraction process, the refreshed samples in the 30 ml broth nutrient medium were diluted into 9 ml broth nutrient sterile 10^{-1} up to 10^{-5} . Each dilutions were grown on a plate count agar medium by incubate them at 37 °C for 2 x 24 hours. After incubating the petri dishes which were contain samples from each dilution, then the colonies bacteria from alga would appear. The colonies bacteria producing antimicrobial compounds were characterized by a clear zone around the colonies. Furthermore, the colonies with stable inhibition zones were collected by isolating them on slant agar medium, with a clear code.

Selection of Symbiont Bacteria Isolates Antagonistically against Pathogenic Bacteria

48 To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria, a qualitative test was
49 conducted directly by scratching round the isolates on the surface of the media that has been dispersed with test bacteria
50 (*Eschericia coli* and *Staphylococcus aureus*). Media were incubated for 48 hours at 37 °C. Each scratching round of isolates
51 was then marked by a unique code.

52 Inhibition zones were determined as those showing clear zones around the colony of symbiont bacteria isolates, for
53 both *Escheriacia coli* and *Staphylococcus aureus* . Strains that showed maximum antagonistic effect against tested pathogens
54 were identified. These were isolated and selected for further antibacterial testing by paper disc and identification of
55 phenotype and genotype .

56 **Antibacterial Potential Testing of Symbiont Bacterial Isolate by Paper Disc Diffusion**

57 Testing the supernatant of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was performed by the
58 agar diffusion method (Hudzicki, 2009)). Supernatant was obtained by separating the filtrate and supernatant bycentrifuge
59 for 1 hour (25 °C and 3000 rpm). Paper discs containing supernatant 40 µL and the negative control nutrient broth 40 µL
60 were left for 1 hour to reduce the water excess, and positive control chloramphenicol 0.01 mg/mL, were placed on the
61 surface of the Mueller Hinton Agar medium containing 1 mL test bacteria and incubated for 48 hours at 37 °C. The
62 supernatant diffuses from the disc into the agar. If the organism is killed or inhibited by both the supernatant and
63 chloramphenicol as antibiotic positive control, there will be no growth in the immediate area around the disc, this is called
64 the zone of inhibition. The zone sizes were compared to assess bioactivity as sensitive, resistant, or intermediate, in each
65 case the resistance zone where shows no colonies growth was measured by using ruler to the nearest mm.

66 **Identification of Phenotype and Genotype of Symbiont Bacteria**

67 General bacterial identification (Lay, 1994 and identification keys from Cowan and Steel (1993)) followed colony
68 characteristic observations on liquid medium and solid medium, observing cell morphology (gram staining, spore staining,
69 and Ziehl-Neelsen staining), and Biochemistry test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase).
70 The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *Turbinaria conoides*
71 samples on the agar medium in pour plating. Observation of medium incubated with temperature 37°C was done at
72 incubation time reached 24 hours and 48 hours. The data obtained from the bacterial isolate characterization were used to
73 estimate the type of symbiotic bacteria isolated from *Turbinaria conoides*. Determination of the type of bacteria was
74 performed based on. Symbiont bacteria species was determined by molecular testing.

75 The DNA of the symbiont bacteria isolates was amplified using primers 9F and 1541R. The DNA bands used were
76 relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf German)
77 with a first predenaturation at 94 ° C for 90 seconds, followed by 30 cycles consisting of denaturation at 95 ° C for 30
78 seconds, primary attachment at 50 ° C for 30 seconds and extension at 72 ° C for 90 seconds. After 30 cycles completed,
79 followed by the elongation phase at 72 ° C for 5 min and cooling at 4 ° C for 20 min. Molecular identification was done
80 through partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES method (Pitcher et al., 1989.
81 Modified). PCR Amplification on 16S rDNA using Primer 9 F: 5`-- AAG GAG GTG ATC CAG CC-3` and Primer 1541
82 R: 5` - GAG TTT GAT CCT GGC TCA G - 3` (White et al., 1990 ; O`Donnell, 1993). The analysis of nitrogen base sequence
83 readings was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems).
84 The next sequenced raw data were trimmed and assembled using the BioEdit program
85 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequencing data were assembled in BLAST with genomic data
86 registered in DDBJ / DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>)

87 **RESULTS AND DISCUSSION**

88 **The Result of Symbiont Bacteria Isolation**

89 Samples consisted of the inside and outside of the algae, as many as 20 petri for 2 replications resulted in colonies
90 with the inhibit zone of 14 colonies, 6 of which were from epibionts, while the other 8 came from the algal tissue. The
91 results of identification of colonies grown on mixed cultures can be seen in Table 1. and identification of isolates isolated
92 into slant agar can be seen in Table 2.

93
94
95
96
97
98
99
100
101
102
103

Tabel 1. Macroscopic forms of bacterial colonies

No	Colony code	Morphology of colonies			
		Shape	Color	Edges	Elevation
1	TUL ² -A1-2	Round	White	Flat	Convex shiny
2	TUL ² -A2-2	Round	White	Flat	Convex shiny
3	TUL ² -A3-2	Round	White	Flat	Convex shiny
4	TUL ² -A4-2	Round	White	Flat	Convex shiny
5	TUL ² -B1-2	Round	White	Crooked	Convex shiny
6	TUL ² -B2-2	Round	White	Crooked	Convex shiny
7	TUD ⁴ -C1-2	Round	White	Flat	Convex shiny
8	TUD ⁴ -C2-2	Round	White	Flat	Convex shiny
9	TUD ² -D1-2	Round	White	Crooked	Convex shiny
10	TUD ² -D2-2	Round	White	Crooked	Convex shiny
11	TUD ² -D3-2	Round	White	Crooked	Convex shiny
12	TUD ² -D4-2	Round	White	Crooked	Convex shiny
13	TUD ⁵ -E-2	Round	White	Flat	Convex shiny
14	TUD ³ -F-2	Round	White	Flat	Convex shiny

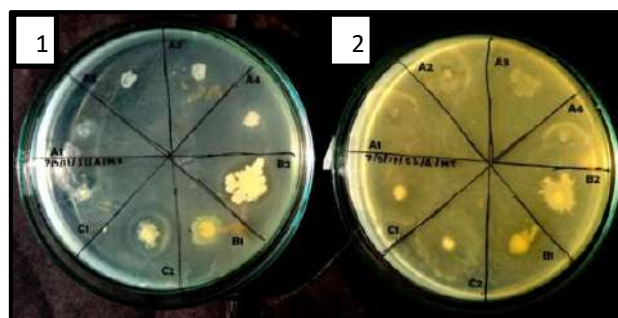
104 Information:
105 *The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
106 ** The code of isolates (²), (⁴), (⁵), (³) states isolates obtained from the dilution
107 *** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the
108 number code representing isolates in the order of the first, second, and so on according the best inhibition zone of each colony observed
109 on the plate
110 **** The code of number 2 identifies the isolate obtained from the second repeat

111 Table 2. Identification of the isolates on slant agar

No	Code of isolates	Solid medium	
		Shape	Color
1.	TUL ² -A1-2	Spread	Milky white
2.	TUL ² -A2-2	Spread	Milky white
3.	TUL ² -A3-2	Spread	Milky white
4.	TUL ² -A4-2	Spread	Milky white
5.	TUL ² -B1-2	Rhizoidal	Cloudy white
6.	TUL ² -B2-2	Rhizoidal	Cloudy white
7.	TUD ⁴ -C1-2	Spread	Milky white
8.	TUD ⁴ -C2-2	Spread	Milky white
9.	TUD ² -D1-2	Rhizoidal	Cloudy white
10.	TUD ² -D2-2	Rhizoidal	Cloudy white
11.	TUD ² -D3-2	Rhizoidal	Cloudy white
12.	TUD ² -D4-2	Rhizoidal	Cloudy white
13.	TUD ⁵ -E-2	Spread	Milky white
14.	TUD ³ -F-2	Spread	Milky white

113
114 Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is different
115 for each species and it is characteristic of a particular species (Dwidjoseputro, 1981).

116 **The Selection Results Symbiont Bacteria Producing Antibacterial Compounds**



117
118 Figure 1. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

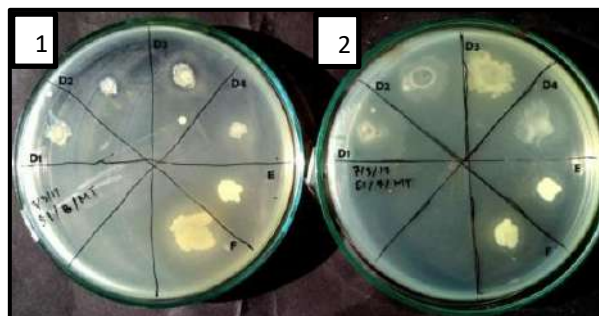


Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167

Based on the results of the direct challenge test, 7 bacterial isolates from 14 isolates tested showed inhibitory activity against *S.aureus* and only 2 of the 7 isolates had inhibitory activity against *E.coli*. The isolate codes that have inhibitory zones against *S.aureus* bacteria are TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2, whereas TUD4-C1-2, And TUD4-C2-2 showed inhibition zones against both pathogenic bacteria being tested but the inhibitory activity against *E.coli* was not as good as its inhibition against *S.aureus*.

Isolates with showing inhibition were re-selected by looking at the best and largest clear zone. Isolates with code TUD4-C2-2 were isolates which had the best inhibition zone. Bacterial isolates derived from tissue showed better inhibition than isolates derived from epibionts. Inhibitory zone and diameter measurement results against *S.aureus* and *E.coli* can be seen in Figure 3 and Table 3. Positive controls have a broader spectrum in inhibiting both types of test bacteria with 16.8 mm inhibition against *S.aureus* and 13.8 mm against *E.coli*. Chloramphenicol with a concentration of 0.03 mg on a paper disc is highly active if its inhibition zone is more than 18 mm (Lay, 1994), while the dose of chloramphenicol (positive control) used is lower at 0.01 mg, so it can be said that bacteria Test is sensitive to positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that a supernatant still containing medium has no effect on the activity formed. From the stability of the measured inhibition zone, the antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram positive bacteria and are merely bacteriostatic for Gram negative bacteria. Paper disc with a supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a 48-hour incubation period. While against Gram negative bacteria, around the disc paper shows the presence of inhibitory activity but gradually become turbid before the incubation period reaches 24 hours.

The sequence of DNA sequencing results of the 16S rDNA encoding of symbiont bacteria isolates showed an accurate score for species level with a similarity of $\geq 99\%$ of the sequences present in GenBank, Then the species homology of the isolates tested was *Lactobacillus plantarum*. Classification of bacterial isolates are *Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus plantarum*.

The antibacterial compounds produced by symbiont bacteria isolates showed different inhibitory activity against both tested bacteria *S.aureus* and *E.coli*. According to Abubakar et al (2011) in Sartika et al (2014) the inner symbiotic bacteria generally have abundant populations and are specific microbes because they directly interact with the bioactive compounds produced from within the algae. While the symbiotic bacteria originating from the surface have a population that is less suspected because it requires higher defense power to overcome the pathogens and predators that are around the algae.



Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Lay, 1994). Other factors that influence the ability of inhibition are the concentration or intensity of antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of organic matter and the degree of acidity (pH) (Sulistijowati and Mile, 2015).

168 Table 3. Results of measurement of inhibitory zone diameter of antibacterial compounds

Repetition	Diameter of zone inhibition (mm)					
	Gram positive			Gram negative		
	Symbiont bacterial (++)	Control (+)	Control (-)	Symbiont bacterial (++)	Control (+)	Control (-)
1	5,5	16	0	0	13,5	0
2	7,8	17,5	0	0	14	0
Average	6,7	16,8	0	0	13,8	0

169 The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Edrada (1998) in
 170 Kusumadewi (2004) a measured inhibition zone of less than 10 mm shows weak activity and strong activity if the the
 171 inhibition zone is greater than 15 mm. Testing of antibacterial activity of the symbiont bacteria supernatant obtained was
 172 still far from the results of the antibiotic activity of the chloramphenicol control. This is because the antibacterial compound
 173 of the extracted symbiont bacteria was a supernatant containing secondary metabolites. However, the test results provide
 174 clear evidence of antibacterial activity. Generally the chemical structure of metabolites from marine products differs from
 175 those of terrestrial origin (Gudbjarnason 1999 in Nofiani, 2005). Seawater contains an active inhibitor agent for Gram
 176 positive bacteria Okami (1982) in Nofiani (2005)

177 **Identification of Phenotype and Genotype of Symbiont Bacteria**

178 Based on phenotypic identification results through cell staining and biochemical testing, symbiont bacteria were rod
 179 shaped, non acidic, non spore forming, non motile, aerobically grown, negative catalase, and positive to carbohydrate tests.
 180 In general, the identification of selected isolates showed specific characteristics of lactic acid bacteria (*Lactobacillus* spp.),
 181 such as round colonies, milky white, Gram positive with short stem cells, without forming endospores (Desniar 2012 in
 182 Saskia, 2014).

183



184

185

Figure 4. Sequens of 16S rDNA

186 Data for base sequence encoding gene of 16S rDNA shows that symbiont bacteria has accurate scores for species
 187 levels with a similarity 100% of the sequences present in GenBank (Figure 4), The species homology of the tested isolate
 188 was *Lactobacillus plantarum*.

189

CONCLUSION

190 *Turbinaria conoides* is commonly found in the gulf of Banten, Serang district, province of Banten. This research
 191 shows that symbiont bacteria *Lactobacillus plantarum* are endophytic and potentially useful as an antibacterial agent against
 192 common pathogens.

193

REFERENCES

194 Cowan ST and Steel KJ. 1993. *Manual for the Identification of Medical Bacteria*. 3rd Edition. University of Cambridge. UK. Dwidjoseputro, D.
 195 1981. *Dasar-dasar Mikrobiologi*. Cetakan ke-5. Djambatan, 1981: Jakarta.
 196 Hudzicki. 2009. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, ASM Microbelibrary. American Society for Microbiology. [http://www.
 197 microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauerdisk-diffusion-susceptibility-test-protocol..](http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauerdisk-diffusion-susceptibility-test-protocol..) (7/04/2014)
 198 Kalaivani G, Hemalatha N, dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria Producing Antagonistic Bioactive
 199 Compounds Against Uti Pathogens*. International Journal of Pharma and Bio Sciences 2016 April; 7(2): (B) 395 – 405. India.
 200 Kusumadewi R. 2004. *Penapisan Awal Senyawa Bioaktif Antibakteri dari Melati Laut (Clerodendrum inerme)*. Skripsi. Fakultas Perikanan dan Ilmu
 201 Kelautan. Institut Pertanian Bogor: Bogor.

- 202 Lay BW. 1994. *Analisis Mikroba di Laboratorium*. PT. Raja Grafindo Persada: Jakarta
- 203 Nofiani R. 2005. *Urgensi dan Mekanisme Biosintesis Metabolit Sekunder Mikroba Laut*. Jurnal Natur Indonesia 10 (2), April 2008: 120-125.
- 204 O'Donnell K. 1993. *Fusarium and its near relatives*. In: Reynolds DR & Taylor JW (Eds) *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic*
- 205 *Speciation in Fungal Systematics* (pp 225–233). CAB International, Wallingford, UK.
- 206 Pitcher DG, Saunders NA, Owen RJ. 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Letters in Applied Microbiology*
- 207 1989, 8: 151-156.
- 208 Sartika, Ahmad A, Natsir, H. 2014. *Potensi Antimikroba Protein Bioaktif dari Bakteri Symbion Alga Coklat Sargassum sp. Asal Perairan Pulau Lae-lae*.
- 209 *Jurnal: FMIPA Universitas Hasanuddin*. Makassar.
- 210 Saskia A. 2014. *Pengembangan Kultur Kering Bakteri Lactobacillus plantarum (SK5) asal Bekasam sebagai Kandidat Probiotik dengan Teknik*
- 211 *Pengeringan Beku*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor: Bogor.
- 212 Siregar AF, Sabdono A, Pringgencies, D. 2012. *Potensi Antibakteri Ekstrak Rumput Laut Terhadap Bakteri Penyakit Kulit Pseudomonas aeruginosa,*
- 213 *Staphylococcus epidermidis, dan Micrococcus*. Journal Of Marine Research. Volume 1, Nomor 2, Tahun 2012, Halaman 152-160.
- 214 Sulistijowati R and Mile L. 2015. *Efektivitas Penghambatan Filtrat Asam Laktat Lactobacillus Sp. Hasil Isolasi Dari Usus Ikan Bandeng (Chanos chanos)*
- 215 *Terhadap Bakteri Patogen*. Fakultas Perikanan dan Ilmu Kelautan Universitas Negeri Gorontalo.
- 216 White TJ, Bruns T, Lee, S. and Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322. In
- 217 *PCR Protocols: A guide to Methods and Applications*, Academic Press, Inc., New York.

218

FILE 2. BUKTI ACCEPTED / TANGGAL 26 DESEMBER 2020**Antibacterial potential of symbiont bacteria of brown algae
(*Turbinaria conoides*) obtained from Indonesian waters**

**NIKEN DHARMAYANTI^{*}, ARMA ANTI, RESMI RUMENTA SIREGAR, YULIATI H. SIPAHUTAR,
AEF PERMADI, ARPAN NASRI SIREGAR, RANDI BOKHI SALAMPESY, SUJULIYANI,
SITI ZACHRO NURBANI, HENI BUDI PURNAMASARI**

Program of Fish Processing Technology, Politeknik Ahli Usaha Perikanan. Jl. Pasar Minggu, South Jakarta 12520, Jakarta, Indonesia,
Tel.: +62-21-7806874, ^{*}email: niken.stp@gmail.com

Manuscript received: 7 October 2020. Revision accepted: 26 December 2020.

Abstract. Dharmayanti N, Anti A, Siregar RR, Sipahutar Y, Permadi A, Siregar AN, Salampesy RB, Sujuliyanti, Nurbani SZ, Purnamasari HB. 2020. Title. Biodiversitas 22: 373-378. Brown seaweeds have the potential to produce bioactive compounds. Bacteria associated with seaweeds are involved in the production of metabolites. Microbes may be present as a living symbiotic in association with other algae as epiphytes or endophytes. In this study, bacteria isolated from brown seaweed (*Turbinaria conoides*) were tested for antibacterial activity. A total of 14 bacteria were isolated, of which 6 were isolated from external tissue, while 8 from internal tissue. Results of an antagonistic test revealed that 7 isolates showed inhibitory activity against *Staphylococcus aureus* and only 1 isolate showed the inhibition against both *S. aureus* and *Escherichia coli*. Phenotypic and genotypic analysis showed that the symbiont bacteria was *Lactobacillus plantarum*.

Keywords: Bioassay, brown seaweed, antagonistic, diffusion paper disc, *Lactobacillus plantarum*

INTRODUCTION

Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse, and unique compounds (Bahare et al. 2019). Many substances are obtained from seaweed, such as alginates, carrageenan, and agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea et al. 2019). Other compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory, and antifouling activity. Therefore, seaweed can provide promising bioactive that can be used in the treatment of human diseases or new antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. Seaweed applications are particularly used in the design of new antimicrobial drugs. Research for the identification of promising algal species, standardization of analytical methods, isolation of compounds through integrated fractionation of bioassays, detailed chemical characterization and evaluation of their safety, evaluation of synergistic effects between components, and efforts to improve yields and lowering extraction costs is needed (Marie et al. 2016).

In later decades, strides microbiological procedures have altogether made a difference in build-up phylogenetic affiliations of seaweed-related epi bacterial communities and endophytes. Be that as it may, there's inadequately

prove that utilitarian connections for seaweed-bacterial intuitive can be built up and well caught on. Epiphytic bacterial communities are rapid colonizers of the ocean growth surface, some of the time versatile and able to quickly metabolize algal exudates (Singh and Reddy, 2014). It has traditionally been used for children's fever, as a fertilizer, repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011). Seaweeds can secrete secondary metabolites with antibacterial properties (Emer and Nissreen 2016). The symbiotic mutualism occurs as algae provide essential sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiont bacteria isolates as algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of infections acquired from the community (Arumugama et al. 2017). *T. conoides* is a tropical marine alga widely distributed in coastal waters in Asia. This study evaluates the properties of the brown alga *Turbinaria conoides* in producing bioactive compounds including the inhibition of human pathogens.

MATERIALS AND METHODS

Procedures

Sampling

Samples of *Turbinaria* sp. (about 1 kg wet weight) were taken from Lima island (S: -6.001051; E: 106.153804). Samples were maintained in fresh seawater for laboratory analysis within 24 hours of collection.

Isolation of symbiont bacteria producing antibacterial compounds

Bacteria were isolated in a solid medium and the size of the colony was different for each species and was characteristic of a particular species (Erin 2012). Epibionts were extracted from 15 grams of algae by rinse in with 30 mL of sterile seawater. The rinse water was incubated in 30 mL of nutrient broth medium and shaken at room temperature for 24 hours. The bioactive compound was extracted by crushing 15 g of alga with a mortar and pestle with the addition of 15 ml of sterile seawater. The suspension was insert into a 30 ml nutrient broth medium and shaken at room temperature for 24 hours.

. After the extraction process, 1 ml of refresh samples were diluted in a 9 ml of sterile nutrient broth to make 10^{-1} dilution. This process was continued to achieve 10^{-5} dilution. Each dilution was grown on a plate count agar medium by incubating them at 37 °C for 2 x 24 hours... Colonies of bacteria that produce antimicrobial compounds were characterized by a clear zone. Furthermore, the colonies with stable inhibition zones were collected by isolating them on a slant agar medium.

Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria

For this, a qualitative test was carried out directly by scratching the isolates on the surface of the media that has been dispersed with two test bacteria, i.e. *Escherichia coli* and *Staphylococcus aureus* (Monte, et al 2014). The media was then incubated for 48 hours at 37 °C. Each scratching round of isolates was then marked by a unique code. Inhibition zones were showing clear zones around the colony of symbiont bacteria isolates for both *Escherichia coli* and *Staphylococcus aureus*. Strains that showed maximum antagonistic effect against tested pathogens were identified. Strains showing maximum antagonistic effects were isolated and selected for antibacterial testing by the paper disc diffusion method. Further, the strains were identified at the phenotypic and genotypic levels.

Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion

Antibacterial testing of symbiont bacteria for inhibitory growth of *E. coli* and *S. aureus* was performed by the paper disc diffusion method (Grela et al. 2018). The supernatant was obtained by separating the filtrate and the supernatant was centrifuged for 1 hour (25 °C and 3000 rpm). Paper discs containing 40 µL supernatant was considered as the treatment while 40 µL nutrient broth was used in negative control and chloramphenicol (0.01 mg/mL) was used as a positive control. After that, the discs were placed on the

surface of the Mueller Hinton Agar medium containing 1 mL test bacteria and incubated for 48 hours at 37 °C. The supernatant diffuses from the disc into the agar. The presence of a clear zone around the supernatant and antibiotic discs was measured by the meter rule in mm. The zone sizes were compared to assess bioactivity as sensitive, resistant, or intermediate, and the presence of a clear zone was measured by the meter rule in mm.

Identification of phenotype and genotype of symbiont bacteria

General bacterial identification based on colony characteristic observations on liquid medium and solid medium followed by cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and biochemical test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase) as described by Phumudzo, (2013). The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *Turbinaria conoides* samples on the agar medium. The plates were incubated at 37°C temperature for 24 to 48 hours. The data obtained from the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from *Turbinaria conoides*. The DNA of the symbiont bacteria isolated was amplified using primers 9F and 1541R. The DNA bands used were relevant to the resulting PCR product of about 1400 base pairs. The PCR reaction used a PCR machine (Eppendorf German) with a first pre-denaturation at 94 ° C for 90 seconds, followed by 30 cycles consisting of denaturation at 95 ° C for 30 seconds, primary attachment at 50 ° C for 30 seconds, and extension at 72 ° C for 90 seconds, followed by the elongation phase at 72 ° C for 5 min and cooling at 4 ° C for 20 min. Molecular identification was done through partial genetic analysis of 16S rDNA. DNA extraction was performed using the GES method (Pitcher et al., 1989 Modified). PCR Amplification on 16S rDNA using Primer 9 F: 5'-- AAG GAG GTG ATC CAG CC-3' and Primer 1541 R: 5' - GAG TTT GAT CCT GGC TCA G - 3' (White et al., 1990, O'Donnell, 1993). The analysis of nitrogen base sequence readings was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data were trimmed and assembled using the BioEdit program (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Sequencing data were assembled in BLAST with genomic data registered in DDBJ / DNA Data Bank of Japan (<http://blast.ddbj.nig.ac.jp/>).

RESULTS AND DISCUSSION

The result of symbiont bacteria isolation

A total of 14 colonies were isolated, of which 6 were from epibionts while the other 8 were from algal tissue. The macroscopic results of colonies on mixed cultures can be seen in Table 1, and on slant agar can be seen in Table 2.

The selection results symbiont bacteria producing antibacterial compounds

Based on the results of the direct challenge test, only 5 bacterial isolates i.e. TUL2-B1-2, TUL2-B2-2, TUD2-D2-2, TUD2-D3-2, and TUD3-F-2 showed inhibitory activity

Table 1. Macroscopic forms of bacterial colonies.

Colony code	Morphology of colonies			
	Shape	Color	Edges	Elevation
TUL ² -A1-2	Round	White	Flat	Convex shiny
TUL ² -A2-2	Round	White	Flat	Convex shiny
TUL ² -A3-2	Round	White	Flat	Convex shiny
TUL ² -A4-2	Round	White	Flat	Convex shiny
TUL ² -B1-2	Round	White	Crooked	Convex shiny
TUL ² -B2-2	Round	White	Crooked	Convex shiny
TUD ⁴ -C1-2	Round	White	Flat	Convex shiny
TUD ⁴ -C2-2	Round	White	Flat	Convex shiny
TUD ² -D1-2	Round	White	Crooked	Convex shiny
TUD ² -D2-2	Round	White	Crooked	Convex shiny
TUD ² -D3-2	Round	White	Crooked	Convex shiny
TUD ² -D4-2	Round	White	Crooked	Convex shiny
TUD ⁵ -E-2	Round	White	Flat	Convex shiny
TUD ³ -F-2	Round	White	Flat	Convex shiny

Note: *The code of isolates TUL/TUD states the isolates originating from the outer/inner algae. ** The code of isolates (²), (⁴), (⁵), (³) states isolates obtained from the dilution. *** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according to the best inhibition zone of each colony observed on the plate. **** The code of number 2 identifies the isolate obtained from the second repeat

against *S.aureus* whereas only 2 viz. TUD4-C1-2 and TUD4-C2-2 bacterial isolates showed inhibition zones against both pathogenic bacteria. The inhibition activity was found to be lower in *E. coli* than in *S. aureus*.

Table 2. Macroscopic form of the isolates on slant agar

Code of isolates	Solid medium	
	Shape	Color
TUL ² -A1-2	Spread	Milky white
TUL ² -A2-2	Spread	Milky white
TUL ² -A3-2	Spread	Milky white
TUL ² -A4-2	Spread	Milky white
TUL ² -B1-2	Rhizoidal	Cloudy white
TUL ² -B2-2	Rhizoidal	Cloudy white
TUD ⁴ -C1-2	Spread	Milky white
TUD ⁴ -C2-2	Spread	Milky white
TUD ² -D1-2	Rhizoidal	Cloudy white
TUD ² -D2-2	Rhizoidal	Cloudy white
TUD ² -D3-2	Rhizoidal	Cloudy white
TUD ² -D4-2	Rhizoidal	Cloudy white
TUD ⁵ -E-2	Spread	Milky white
TUD ³ -F-2	Spread	Milky white

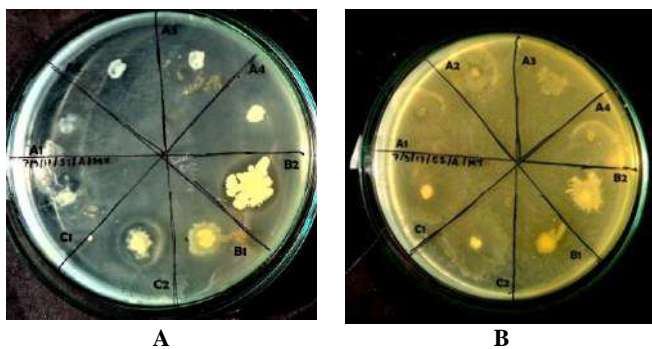


Figure 1. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

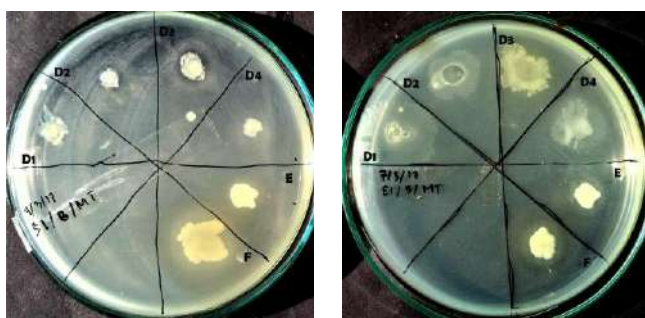


Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

Isolates with code TUD4-C2-2 had the best inhibition zone. Bacterial isolates derived from algal tissue showed better inhibition than isolates derived from epibionts. The inhibitory zone and diameter measurement results against *S. aureus* and *E.coli* can be seen in Figure 3 and Table 3. Positive controls showed 16.8 mm inhibition zone against *S. aureus* and 13.8 mm inhibition zone against *E. coli*. Chloramphenicol with a concentration of 0.03 mg on a paper disc is highly active if its inhibition zone is more than 18 mm (Mounyr et al., 2016), while the dose of chloramphenicol (positive control) used was less than 0.01 mg, so it can be said that bacteria test was found to be sensitive to the positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that supernatant does not affect the activity formed. The antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram-positive bacteria and were merely bacteriostatic for Gram-negative bacteria. As gram-positive symbiotic bacteria widely know contain bacteriocins (Mezaini A et al, 2009 and Li D. Et al, 2015) bacteriocins from Gram-

positive bacteria are generally not effective against Gram-negative bacteria (Smaoui et al, 2010). Paper disc with supernatant applied to a Gram-positive bacterial plate indicate a stable clear zone even after a 48-hour incubation period. While against the Gram-negative bacteria, the presence of inhibitory activity appeared around the disc paper, but it was gradually turbulent before the incubation period reaches 24 hours. The antibacterial compounds produced by symbiont bacterial isolates showed different inhibitory activity against both tested bacteria *S.aureus* and *E.coli*. According to Irma et al. (2011), the inner symbiotic bacteria generally have abundant populations and are specific because they directly interact with the bioactive compounds produced from within the algae. While the symbiotic bacteria were less populated, as it required higher defense power to overcome the pathogens and predators that are around the algae.

Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Fernando and Bruce, 2020). Other factors that affect the inhibition potential are the concentration or intensity of antimicrobial agents, the number of microorganisms, the temperature, the species of microorganisms, the presence of organic matter, and the degree of acidity (pH) (Manisha and Shyamapada 2011).

The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Mounyr et al (2016), less than 10 mm inhibition zone showed weak activity and if the inhibition zone is greater than 15 mm it indicates strong activity. Testing of antibacterial activity of the symbiont bacteria supernatant obtained was still far from the results of the antibiotic activity of the chloramphenicol control. This is because of the supernatant containing secondary metabolites. However, the test results provide clear evidence of antibacterial activity. Generally, the chemical structure of metabolites from marine products

differs from the terrestrial origin. Marine bacteria are significant reservoirs of bioactive molecules that have never been found in terrestrial organisms (Giovanna, 2020). Seawater contains an active inhibitor agent for Gram-positive bacteria (Kapoor et al. 2017).

Identification of phenotype and genotype of symbiont bacteria

The known characteristics of symbiont bacteria through phenotypic observation and biochemical tests include rod-shaped, non-acidic, non-spore-forming, non-motile, grow aerobically, negative catalase, and positive carbohydrate test. In general, the selected isolate showed special characteristics possessed by lactic acid bacteria (*Lactobacillus* spp.), such as circular, smooth white, Gram-positive colonies with brief stem cells, without shaping endospores (Davoodabadi et al. 2015).

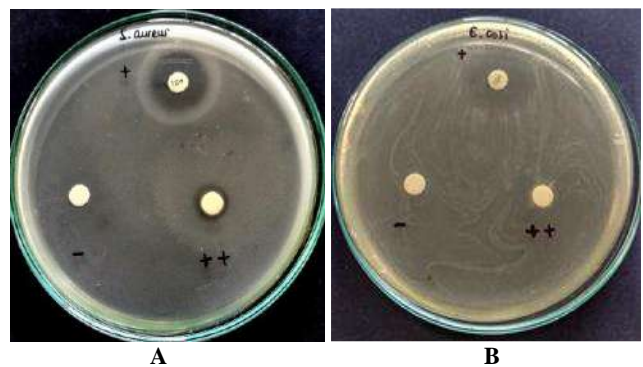


Figure 3. Results of antibiotic susceptibility test against: A. *Staphylococcus aureus* and B. *Escherichia coli*

Table 3. Results of inhibitory zone diameter

Repetition	The diameter of zone inhibition (mm)					
	Gram-positive			Gram-negative		
	Symbiont bacterial	Control	Control	Symbiont bacterial	Control	Control
	(++)	(+)	(-)	(++)	(+)	(-)
1	5.5	16	0	0	13.5	0
2	7.8	17.5	0	0	14	0
Average	6.7	16.8	0	0	13.8	0

Sequens of 16S rDNA

GCTCAGGACGAAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCCTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCAGAAAGCGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAGATGGCTTCGGCTATCACCTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGCTCACATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACCTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCTGAGTGAAGAAGGGTTTCGGCTCGTAAAACCTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACCTGTTACGATATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCACTGGCGAAGGCGGCTGTCTGGTCTGTAACCTGACGCTGAGGCTCGAAAATATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCCTAAACGATGAATGCTAAGTGTGGAGGGTTTCGGCCCTTCAGTGCCTGACGCTAACGCATTAAGCATTCCGCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTGAGTCTGCTGAGATGTTGG

GTAAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGA
GTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC
ATGCCGCGGTGAATACGTTCCCGGGCCTTGACACACCGCCCGTACACCATGAGAGTTTGTAAACCCCAAAGTC

Figure 4. The arrangement of nitrogen bases sequenced from symbiont bacteria, A = adenine, T = thiamine, G = guanine, C = cytosine.

The molecular identification was done through partial genetic analysis of 16S rDNA. PCR amplification results from the 16S region of bacterial ribosome DNA Nitrogen base sequences sorted from symbiont bacterial isolates can be seen in Figure 4. The sequencing information was under the influence of genomic information enlisted within the DDBJ / Japanese DNA Information Bank with 100% strains grouping comes about. The characters of the antibacterial strains were indistinguishable from those of *Lactobacillus plantarum*. The highest 100% personality, greatest score 2660, add up to score 2660, 100% inquiry scope, E esteem 0, was recorded for the taxon of adjacent microbes. The classification of the bacterial isolate is Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus plantarum.

Data for base sequence encoding gene of 16S rDNA shows that symbiont bacteria have accurate scores for species levels with a similarity of 100% of the sequences present in GenBank. The species homology of the tested isolate was *Lactobacillus plantarum*. *Lactobacillus plantarum* strains separated from dairy items appeared solid antimicrobial action against the pointers strains of *Staphylococcus aureus*, *Salmonella* spp, and *Escherichia coli* (Hu et al 2019). The isolation of *L. plantarum* from Tibetan yaks was able to restrain the development of *E. coli* and *S. aureus* (Wang et al 2018). Some *Lactobacillus* strains showed antibacterial movement against Enterobacteriaceae that were safe for carbapenems (CRE). This effect may have potential applications through the utilize of the *Lactobacillus* strain as a starter culture in aged nourishments or as a nourishment additive to control or avoid CRE contamination (Chen et al 2019).

In conclusion, *Turbinaria conoides* was commonly found in the gulf of Banten, Serang district, province of Banten. This research revealed that symbiont bacteria *Lactobacillus plantarum* was endophytic and potentially useful as an antibacterial agent against common pathogens.

ACKNOWLEDGEMENTS

The research behind this paper would not have been possible without the exceptional support by Jakarta Technical Fisheries University under the Applied Research Program of Fish Processing Technology Study Program. The authors thank the Jakarta Technical Fisheries University for providing scientific publication funding.

REFERENCES

Andrea GZ, Miguel A. Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara, 2019. The potential of seaweeds as a source of functional

- ingredients of prebiotic and antioxidant value. *Antioxid (Basel)* 8(9): 406.
- Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer properties of *Turbinaria conoides* (J. Agardh). *Clin Phytosci* 3: 5
- Bahare S, Javad SR, Ana ML, Seca, Diana CGA, Pinto, Izabela M, Antonio T, Abhay PM, Manisha N, Wissam Z, Natália M, 2019. Current trends on seaweeds: looking at chemical composition, phytopharmacology, and cosmetic applications. *Mol.* 24 (22): 4182.
- Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, Chuang YC, Lu YC, and Tang HJ, 2019. Antimicrobial Activity of *Lactobacillus* Species Against Carbapenem-Resistant Enterobacteriaceae. *Front Microbiol* 10:789
- Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015. Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. *Publish in Anaerobe* 34: 53-58.
- Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological mechanisms and Applications. *Mar Drugs* 14 (4): 81.
- Erin R sanders. 2012. Aseptic Laboratory Techniques: Plating Methods. *J Vis Exp.* 63: 3064
- Baquero F, Levin BR.. 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat Rev Microbiol* (2020)
- Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar Environ Res* (2016)
- Grela E, Kozłowska J, Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem* 120 (4): 303-311
- Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol* 22: 315-326.
- Hu CH, Ren LQ, Zhou Y, Ye BC. 2019. Characterization of antimicrobial activity of three *Lactobacillus plantarum* strains isolated from Chinese traditional dairy food. *Food science & Nutrition* 7 (6): 1997–2005. DOI: 10.1002/fsn3.1025
- Irma ESM, Gómez LJ, Rivas GG, and Sánchez NEA, 2011. Bioactive Compounds from Bacteria Associated to Marine in Algae. *Biotechnology. Molecular Studies and Novel Applications for Improved Quality of Human Life*, Reda Helmy Sammour, IntechOpen. DOI: 10.5772/27842, <https://www.intechopen.com/books/biotechnology-molecular-studies-and-novel-applications-for-improved-quality-of-human-life/bioactive-compounds-from-bacteria-associated-to-marine-algae>
- Kalaivani G, Hemalatha N, Poongothai. 2016. Screening Of Marinene Brown Algae Associated Potential Bacteria Producing Antagonistic Bioactive Compounds Against Uti Pathogens. *International Journal of Pharma and Bio Sci* 7 (2): 395–405.
- Garima K, Saurabh S, Ashok E. 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. *J Anaesthesiol Clin Pharmacol.* 33 (3): 300-305.
- Li D, Ni K, Pang H, Wang Y, Cai Y, Jin Q. 2015. Identification and Antimicrobial Activity Detection of Lactic Acid Bacteria Isolated from Corn Stover Silage. *Asian-Australas J Anim Sci.* 2015 May; 28(5): 620–631.
- Manisha DM and Shyamapada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed.* 2011 Apr; 1(2): 154–160.
- Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs.* 2016 Mar; 14(3): 52.
- Mezaini A, Chihib N E, Bouras A D, Arroume N N, Homez J P. 2009. Antibacterial Activity of Some Lactic Acid Bacteria Isolated from an Algerian Dairy Product. *Journal of Environmental and Public Health.* Volume 2009.
- Monte, J., Abreu, A. C., Borges, A., Simões, L. C., & Simões, M. (2014). Antimicrobial Activity of Selected Phytochemicals against

- Escherichia coli and Staphylococcus aureus and Their Biofilms. *Pathogens (Basel, Switzerland)*, 3(2), 473–498.
- Mounyr B, Moulay S, Saad KI. 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal.* 6 (2): 71-79.
- O'Donnell. 1993. *Fusarium and its Near Relatives*. National Center for Agriculture Utilization Research, USDA, ARS, 1815 N. University Street, Peoria, Illinois, 61604, USA.
- Phumudzo T, Ronald N, Khayaletu N, Fhatuwani M. 2013. Bacterial species identification getting easier. *Afr J Biotechnol* 12 (41): 5975-5982
- Singh RP, Reddy CRK. 2014. Seaweed-microbial interactions: key functions of seaweed-associated bacteria. *FEMS Microbiol. Ecol* 88 (2): 213-230.
- Smaoui S, Elleuch L, Bejar W, Karray-Rebai I, Ayadi I, Jaouadi B, Mathieu F, Chouayekh H, Bejar S, Mellouli L. 2010. Inhibition of fungi and gram-negative bacteria by bacteriocin BacTN635 produced by *Lactobacillus plantarum* sp. TN635. *Appl Biochem Biotechnol.* 162 (4): 1132-46.
- Wang L, Zhang H, Rehman M.U, Khalid Mehmood K, Jiang X, Iqbal M, Tong X, Gao X, Li J,2018. Antibacterial activity of *Lactobacillus plantarum* isolated from Tibetan yaks. *J Microbial Pathogenesis*115: 293-298.
- White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A guide to Methods and Applications*, Academic Press, Inc., New York.

FILE 3. PERTANYAAN/PERINTAH PERBAIKAN DARI PARA REVIEWER JURNAL

3. file pertanyaan/perintah perbaikan dari para reviewer jurnal (bisa beberapa kali, lengkap dengan informasi tanggal) serta jawaban dari pengarang korespondensi Agar klarifikasi disusun secara sistematis, seperti perintah di atas.

Editor/ Reviewer	Date	Reviewer Suggestion	Author response
Editor	9-10-2020	<p>Dear author, Thank you very much for your manuscript submission. Unfortunately, your manuscript does not meet our requirements:</p> <ul style="list-style-type: none"> - At least, to published in the Biodiversitas journal, you need to compose a minimum of 20 references which 80% of international scientific journals published in the last 10 years (2010-2020), and maximum 10% references in the local language (not English). And please write the references based on the author's guidelines. -This manuscript is too brief to be published in the Biodiversitas journal. At least, you need to compose a 2000 words article from the introduction to a conclusion (table and figure are excluded). <p>Kindly check and correct accordingly Thank you</p>	<p>The number of references has been added to 27 libraries which originally amounted to 13 libraries. The number of 27 libraries has exceeded the minimum limit of 20 libraries. Literature composition consists of international journals of science for the last 10 years (2010-2020) as many as 23 libraries or 85% and 0% of local languages meet a minimum of 10% of local languages (not English)</p> <p>The number of words has been added to 2208 words outside Tables and Figures that have exceeded the minimum limit of 2000 words The addition of these words is done by developing a discussion using reference references.</p>
Reviewer B:	9-11-2020	<p>Dear authors of the manuscript: Antibacterial potential of symbiont bacteria of brown algae (<i>Turbinaria conoides</i>) obtained from Indonesian waters. The manuscript focus on symbionts from this macroalga, which may present antibacterial properties. It was with pleasure that I read the work. The study seems relevant, interesting, and important. Nowadays the research of new antimicrobial compounds is crucial and it is known that the sea is a reservoir of this kind of compound. Although I think the outputs selected for the study were</p>	<p>The paper has been improved as requested by adding a discussion of both macroalgae and symbiotic bacteria using libraries from several recent journals</p>

		<p>relevant, I believe you could be more audacious and made other assays to prove antimicrobial activity. I would also like to see results using the macroalgae itself since it is also a pool antioxidant and antimicrobial compounds. Even so, is interesting the symbiotic bacteria that you have found. The results could be better presented and the quality should be improved, or presented in a different way. In attach, I send the manuscript with minor comments for the authors' consideration. Recommendation: Resubmit for Review</p>	
Reviewer F:	9-11-2020	<p>The paper in question concerns itself with isolating bacterial symbiotes of <i>T. conoides</i> and studying their antibacterial activity. In my opinion, the work itself is performed on an acceptable level, but the paper requires some corrections: 1. The text needs careful editing. For example, lines 31-32 go like this: <i>Turbinaria conoides</i> belongs to the family of The recent scientific trends target the pursuit for phytochemicals from marine algae due to their numerous health-promoting effects, pathogens (Mark LW et al. 2016). Obviously, the alga in question does not belong to the family of recent scientific trends, it belongs to the family Sargassaceae. This part was probably copy-pasted incorrectly, and "due to" is in bold for no apparent reasons. There are other typos and similar issues, eg line 28 includes a mention of "bacteri associated with seaweed", while it should be "bacteria". 2. The sequence of antibacterial strain is only provided as an image. I would prefer it to be deposited to Genbank/DBJ or, at the very least, included as plaintext in the paper. The screenshot in fig. 4 does not permit copying sequence for some analysis the readers might want to run. Even despite 100% identity to other published sequences, the fact that the strain from Lima island is identical to those isolated</p>	<p>The paper has made improvements as requested by changing reference sources and discussions. Writing improvements have been made both lines 31-32 as well as lines 28. Figure 4 has been replaced not in the form of an image but returned to the DNA chain arrangement according to the sequencing results. The proposed references have been adjusted for discussion.</p>

		<p>from elsewhere may be important for someone interested in the distribution and diversity of <i>Lactobacillus</i>. 3. <i>Lactobacillus plantarum</i> is well-known for its antibacterial activity, see eg: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6593389/ https://www.sciencedirect.com/science/article/abs/pii/S0882401017314559 https://www.frontiersin.org/articles/10.3389/fmicb.2019.00789/full I think the dicussion and conclusions would benefit from referencing this fact. Should these issues be fixed, I believe the paper will be acceptable for publication. Recommendation: Revisions Required</p>	
Reviewer A:	11-12-2022	<ol style="list-style-type: none"> 1. You did not mention direct test in the method but you wrote it your results. 2. You wrote the results but you did not discuss it with other results add some references. 3. You isolated 14 isolates. Were they all from <i>Lactobacillus</i>. 4. Write references as per the rule of journal norm there are so many mistakes. 5. Some references are missing in the reference so please check it. 	<p>Paper has been improved as follows:</p> <ol style="list-style-type: none"> 1. In the methods section, it is clear about how to test the results in the Results and discussion section. 2. In the results and discussion section, discussion has been improved including the results of other studies based on references from the last 10 years (2010 – 2020). 3. The authors obtained 14 isolates of symbiont bacteria, one symbiont with the best inhibitory zone against <i>coli</i> bacteria to be tested for DNA sequencing and sequencing results as <i>Lactobacillus plantarum</i>. 4. Bibliography writing has been improved according to the rules of writing a Library (APA). 5. Already fixed missing Library writes,