

Submission

The screenshot shows the 'Submission' page of the Biodiversitas Journal of Biological Diversity. At the top, there are tabs for 'Workflow' and 'Publication'. Below them, there are sub-tabs: 'Submission', 'Review', 'Copyediting', and 'Production'. The 'Submission' tab is selected. Under 'Submission Files', there is a list with one item: '30440-1 nikendharmayanti_20201008-Niken-Biodiversitas.docx' (October 7, 2020). A 'Search' button is also present. Below this, the 'Pre-Review Discussions' section shows two entries: 'Comments for the Editor' (from nikendharmayanti, 2020-10-07 11:14 AM) and 'Manuscript Submission' (from ayu, 2020-10-09 05:26 AM). A 'Download All Files' button is located at the bottom of this section. The taskbar at the bottom of the screen shows various open applications like Jurnal Kelautan, Gmail, WhatsApp, and a browser window.

Perbaikan Editor

The screenshot shows the 'Manuscript Submission' interface. At the top, it says 'Participants' and lists 'Assalamualaikum Niken - Dharmayanti -est (nikendharmayant)' and 'Ayu Astuti (ayu)'. Below this is the 'Messages' section. It contains a note from 'ayu' dated '2020-10-09 05:26 AM' stating: '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.' Below this is another message from 'nikendharmayanti' dated '2020-10-12 04:19' stating: 'Assalamualaikum wr. wb.
Dear Editor'. The taskbar at the bottom shows various open applications like Jurnal Kelautan, Gmail, WhatsApp, and a browser window.

Review 1

Dear Editor-in-Chief,

I herewith enclosed a research article.

Title:

Antibacterial 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 Technical 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 manuscript 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: 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

Antibacterial Potential Symbiont Bacteria of Brown Algae (*Turbinaria conoides*) Obtained from Banten Bay Serang District - Province Of Banten Indonesian Waters.

Niken Dharmayanti, Aef Permadi, Yuliati H Sipahutar, Resmi Rumenta Siregar, Arpan Nasri Siregar, Randi Bokhi Salampessy, Sujulyani, 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 symbiont 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* 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 (*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 potential of being a producer 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 (Burgesset 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 alga widely distributed in coastal waters in SE Asia. We chose this alga following extensive trials on other common macroalgae including *Sargassum* spp. and *Euchema 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

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

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

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

42 **MethodsProcedures**

43 **Sampling**

44 Samples of *Turbinaria sp.* (about 1 kg wet weight) was-were taken from Lima island (S: -6.001051°E; E:
45 106.153804) around 1 kg for determination in the morning around 7 at low tide allows the position of algae 1 meter below
46 the water on the sidelines of the reef directly dried in the bundle of the island. Sampling was continued at 16 o'clock when
47 the low tide and taked 500 grams for antibacterial test and kept in the plastic pouch and immediately filled with seawater,
48 with seaweed : water ratio of 1:2 until submerged, when it already arrived on Serang filled with oxygen 1 : 2 more air. The
49 seaweed stayed in the plastic with oxygen from Serang until Jakarta for a night and started done in the laboratorium in the
50 morning.Samples were maintained in fresh seawater for laboratory analyses within 24 hour of collection.

51 **Identification and Determination of Macroalgae**

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

57 **Isolation of Symbiont Bacteria Producing Antibacterial Compounds**

58 Surface of Algae: Epibionts were extracted from 15 grams of algae by rinsed-rinsin with 30 mL of sterile sea water.
59 The rinse water is was put into incubated in 30 mL of nutrient broth medium then shaken by shaker at room temperature for
60 24 hours. Inside of algae: as many as Bioactive compound 15 grams of algae were rinsed with 30 mL of sterile sea water, were
61 extracted by crushed-crushing 15 g of alga finely using mortal with a mortar and pestle with the addition of 15 ml of sterile
62 seawater. The suspension is then fed was incubated into-with 30 mL broth nutrient medium and shaken by shaker att room
63 temperature for 24 hours.

64 After extraction process, The refreshed samples of-in the 30 ml broth nutrient medium were diluted into 9 ml broth
65 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
66 for 2 x 24 hours. After incubating the petri dishes which were contain samples from each dilution, then the colonies bacteria
67 from alga would appear. The colonies bacteria producing antimicrobial compounds are-were characterized by a clear zone
68 around the colonies. Furthermore, the colonies with stable inhibition zones were collected by and isolating themed on
69 slant agar medium, with a clear code.

70 **Selection of Symbiont Bacteria Isolates Antagonistically against Pathogenic Bacteria**

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

75 Inhibition zones were read as the pointdetermined as those showing clear zones around the colony of symbiont
76 bacteria isolates, the more clear zone of isolates in inhibit for both *Escheracia coli* and *Staphylococcus aureus* are the better
77 their activity. Strains -that showed maximum antagonistic effect againsts tested pathogens were choosen and marked by its
78 eodeidentified. Isolates that These choosen isolate with appropriate code which was formed a clear zone or has with the a
79 highest activity are-waswere isolated and selected for further antibacterial testing by paper disc and identification of
80 phenotype and genotype testin.g

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

82 Testing inhibitory the supernatant of symbiont bacteria on thefor inhibitory growth of *E.coli* and *S.aureus* was
83 performed by the agar diffusion method-(Hudzicki, 2009) REFERENCE. Supernatant was obtained by separating the
84 filtrate and supernatant by centrifugation processcentrifuge for 1 hour, -temperature at(25 °C and 3000 rpm). Paper discs
85 containing supernatant 40 µL and the negative control nutrient broth 40 µL which has allowed were dried left for 1 hour to
86 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: 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 2448 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, to 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 Symbiont 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). Symbiont bacteria species was determined by molecular testing.

The DNA of the symbiont 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 was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data was 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

132
133
134
135
136
137
138
139

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 s 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.

140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156

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 ¹ -C1-2	Round	White	Flat	Convex shiny
8	TUD ¹ -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 ¹ -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), (1) 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 the best inhibition zone of each colony observed on the plate

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

157
158
159
160
161
162
163
164
165

Table 22. 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: Left

Formatted Table

Formatted: Indent: Hanging: 1,46 cm

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

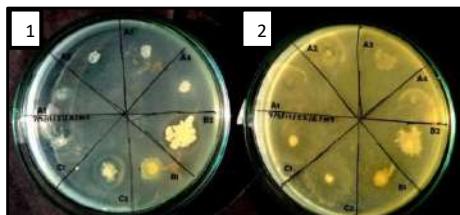
166

167 Observations of bacteria can be done individually or in groups in the form of colonies. If the **b**Bacteria is isolated into a solid
 168 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
 169 a particular species (Dwidjoseputro, 1981).

170

171 The Selection Results Symbiont Bacteria Producing Antibacterial Compounds

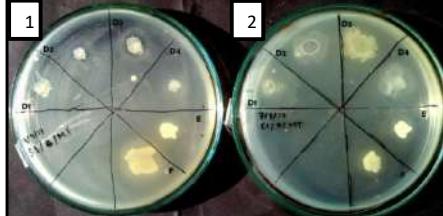
172



173 Figure 12. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

174

175



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

177

178

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

184 Symbioyte bacterial isolates with a specific code that has a resistor zone are re-selected by looking at the best and
 185 largest clear zone. From the observation result, it was determined that isolates with code TUD4 C2 2 were isolates which
 186 had the best inhibition zone. Based on the code given, it is known that this isolate was obtained from the algae's inner sample,
 187 at 10⁴ dilution, the second colony of the isolated third plate, and a colony obtained in the second repetition. Isolates with a
 188 specific code that has a showing inhibition zone were re-selected by looking at the best and largest clear zone. Isolates with
 189 code TUD4-C2-2 were isolates which had the best inhibition zone. From the observation result, it was determined that
 190 isolates with code TUD4-C2-2 were isolates which had the best inhibition zone. Based on the code given, it is known that
 191 this isolate was obtained from the algae's inner sample, at 10⁴ dilution, the second colony of the isolated third plate, and a
 192 colony obtained in the second repetition.

Formatted

Bacterial isolates derived from the insidetissue showed have better activity**better inhibition** than bacterial_isolates derived from the surfaceepibionts. 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 (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.

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.

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 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 $\geq 99\%$ 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.

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 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.

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.

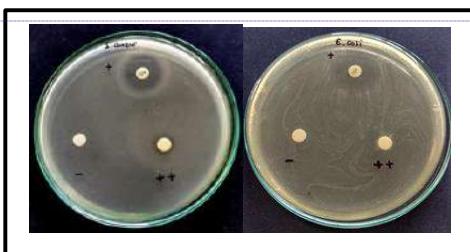


Figure 43. Results of antibiotic susceptibility test against S.aureus and E.coli

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

294 tests. In general, the identification of microscopically selected isolates showed specific characteristics possessed by of
295 lactic acid bacteria (*Lactobacillus* spp.), such as round colonies, milky white, Gram positive with short stem cells, and
296 does without forming endospores (Desnair 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).

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*.

Lactobacillus plantarum_100%

```
GCTCAGGACGAACGCTGGCGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGTGATCATGATTTACAT  
TTGAGTGACTGGCAACTGTGAGTAACACGTGGAAACCTGCCAGAACGGGGATAACACCTGGAAACAGATGCTAATACCG  
CATACAACATTGGGACATGGTCCAGCTGAGTAACAGTGGATGCTTCGCTACTCTTGGATGCTGGCCGGCGTATTAGCTAGATG  
GTGGGTAACCGCTACCATGGCAATGATACGTAGCCACCTGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAA  
CTCTTACGGAGGAGCAGTAGGAAATCTTCCACATGGACGAAAGCTGTGAGGCAACGCCGGTGTAGTAAGAAGGGTTT  
GGCTGTAACACTCTGGTTAAAGAAGAACATATCTAGAGTAACAGTGGCTAGGTGCTGGGATTATTGGCGTAAAGCGAGGCCACGGCTA  
ACTACGTGCCAGCACCGCGGTAATACGTAGGTGCAAGCGTGTCCGGATTATTGGCGTAAAGCGAGGCCACGGCTT  
AAGCTGATGTGAAAGCCTCGGCTCAACCGAAGAAGTGCATCGGAAACTTGGGAAACTTGGAGTGCGAGAAGAGGACAGTGGAACTC  
CATGTTAGCGGTGAAATTCGAGATATATGGAAAGAACCCAGTGGCTGAGCTGGTCTGGTGTGTAAGTACGACGCTGAGGCTC  
GAAAGATATGGTAGCAAACAGGATTAGATACCTGGTAGTGTCCATACCGTAAACGATGAAATGCTAAGTGTGAGGGTTCCGGCCCT  
TCAGTGTGCAAGCTAACGCTTCCGGCTGGGAGTACGGCGCAAGGCTGAAACTCTAAAGGAATTGACGGGGCCCG  
CACAGCGGGAGCATGGTACAGCTGGTGTGCAAGCTGGTGTGAGATGGGGTAAGTCCGGCAACCGGAGGAAGGTGGGATG  
AGCGCAACCCATTATCAGTGGCACGCTTAAAGTGGGACTCTGGTGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGATG  
ACGCTAAATCATCGCCCTTATGACCTGGGCTACACAGTGTCAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAG  
CTAATCTTAAAGCCATTCTCGGATTGTAGCTGCAACTCCCTCATGAAGTCTGGAATCGCTAGTAATCGGGATCAGC  
ATGCCGGTGAACAGTCCGGCCTGTACACACCCTGCACCATGAGAGTTGTAACCCCCAAAGTC
```

Figure 4. Sequens of 16S rDNA

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

4. CONCLUSION

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

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

REFERENCES

- 325 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
326 Anamaya Publishers, New Delhi, India.
327 Cowan, S-T and Steel, K-J. 1993. *Manual for the Identification of Medical Bacteria*. 3rd Edition. University of Cambridge. UK.
328 Dwidjoseputro, D. 1981. *Dasar-dasar Mikrobiologi*. Cetakan ke-5. Djambatan, 1981: Jakarta.
329 Hudzicki, 2009. *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol*. ASM Microbelibrary, American Society for Microbiology. <http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauerdisk-diffusion-susceptibility-test-protocol>, (7/04/2014)

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

Commented [p23]: Moved to Materials and Methods

Commented [p24]: Moved to Results

Formatted: Font: (Default) Arial, Bold

Formatted: Left

Formatted: Font: 9 pt

Formatted: Centered

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Italic

Formatted: Font: 10 pt

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.

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

249 From the stability of the measured inhibition zone, in general the antibacterial properties of the supernatant
250 produced by the symbiotic bacteria act as bactericidal against Gram positive bacteria and are merely bacteriostatic in Gram
251 negative. Paper disc with a supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a
252 48-hour incubation period. While against Gram negative bacteria, around the disc paper shows the presence of inhibitory
253 activity but gradually become turbid before the incubation period reaches 24 hours. Antimicrobial agents may be
254 bacteriostatic at low concentrations but are bactericidal at high concentrations (Lay, 1994). Other factors that influence the
255 ability of inhibitory inhibition are the concentration or intensity of antimicrobial agents, the number of microorganisms, the
256 temperature, the species of microorganisms, the presence of organic matter and the degree of acidity (pH) (Sulistijowati and
257 Mile, 2015).

Formatted: Font: 10 pt

258 Table 34. Results of measurement of inhibitory zone diameter of antibacterial compounds
259

Repetition	Symbiont bacterial (++)	Diameter of zone inhibition (mm)			Gram negative	
		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

260 The area of the symptomatic supernatant inhibition zone of *S.aureus* is was 6.7 mm. According to Edrada (1998) in
261 Kusumadewi (2004) a measured inhibition zone of less than 10 mm belongs to a very shows weak and very active activity if
262 and strong activity if the inhibition zone is greater than 15 mm. Testing of antibacterial activity of the symbiont bacteria
263 supernatant obtained was still far from the results of the antibiotic activity of the tested ecomparatochloramphenicol f control.
264 This is because the antibacterial compound of the applied extracted symbiont bacteria is stillwas a supernatant with
265 thecontaining secondary metabolites. it contains, butHowever, the test results have indicated the presence of provide clear
266 evidence of antibacterial activity. Generally the chemical structure of metabolites from marine products is often different
267 from the secondary differs from those of terrestrial origin metabolite of land (Gudbjarnason 1999 in Nofiani, 2005).
268 Seawater contains an active inhibitor agent for Gram positive bacteria, according to Okami (1982) in in Nofiani (2005)
269 that seawater contains an active inhibitor agent for organisms, seawater has the ability of inhibitors a gainst Gram positive
270 bacteria.
271

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

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

280 Identification of Phenotype and Genotype of Symbiont Bacteria

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

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

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

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Commented [p21]: Moved to Introduction.

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Indent: First line: 0 cm

Commented [p22]: Not directly relevant

Formatted: Font: 10 pt

- 335 Ingratubun, J. A., Ijong, F. G., dan Onibala, H. 2013. *Isolasi dan Identifikasi Bakteri Asam Laktat pada Bakarang sebagai Starter Mikroba Produk*
 336 *Fermentasi*. Jurnal Aquatic Science & Management, Edisi Khusus 1, 48-56. Pascasarjana, Universitas Sam Ratulangi.
- 337 Kalaivani, G., Hemalatha, N., dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria Producing Antagonistic*
 338 *Bioactive Compounds Against Uri Pathogens*. International Journal of Pharma and Bio Sciences 2016 April; 7(2); (B) 395 – 405. India.
- 339 Kusumadewi, R. 2004. *Penapisan Awal Senyawa Bioaktif Antibakteri dari Melati Laut (Clerodendrum inerme)*. Skripsi. Fakultas Perikanan dan Ilmu
 340 Kelautan, Institut Pertanian Bogor: Bogor.
- 341 Lay, B-W. 1994. *Analisis Mikroba di Laboratorium*. PT. Raja Grafindo Persada: Jakarta.
- 342 Lukman, J.B., Dwyanza Z., Raya, I., Priasmabode, D. 2015. *Efektivitas Ekstrak Alga Eucheuma Cottonii, Turbinaria Decurrens, dan Ulva Reticulata*
 343 *Sebagai Antimikroba terhadap Streptococcus Mutans*. Jurnal Jurusan Biologi FMIPA Universitas Hasanuddin: Makassar.
- 344 Nofiani, R. 2005. *Urgensi dan Mekanisme Biosintesis Metabolit Sekunder Mikroba Laut*. Jurnal Natur Indonesia 10 (2), April 2008: 120-125.
- 345 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.
- 346 Pitcher DG, Saunders NA, Owen RJ. 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Letters in Applied Microbiology* 8: 151-156.
- 347 Pelezar, M.J dan Chan, E.C.S. 1986. *Dasar dasar Mikrobiologi*. Diterjemahkan oleh Ratnasari, dkk. Edisi 1. UI Press, Jakarta.
- 348 Sahara, F. N. I., Radjaea, O., K. dan Supriyatini, E. 2013. *Identifikasi Pigmen Karotenoid pada Bakteri Simbion Rumput Laut Kappaphycus alvarezii*.
 349 *Journal Of Marine Research*, Volume 2, Nomor 3, Tahun 2013, Halaman 58-67. Online di: <http://ejournal.s1.undip.ac.id/index.php/jmr>.
- 350 Sartika, Ahmad, A., dan Natsir, H. 2014. *Potensi Antimikroba Protein Bioaktif dari Bakteri Simbion Alga Coklat Sargassum sp. Asal Perairan Pulau Lae-
 351 lae*. Jurnal: FMIPA Universitas Hasanuddin: Makassar.
- 352 Saskia, A. 2014. *Pengembangan Kultur Kering Bakteri Lactobacillus plantarum (SK5) asal Bekasan sebagai Kandidat Probiotik dengan Teknik*
 353 *Pengerinan Beku*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor: Bogor.
- 354 Siregar, A.-F., Sabdono, A., dan Pringgenies, D. 2012. *Potensi Antibakteri Ekstrak Rumput Laut Terhadap Bakteri Penyakit Kulit Pseudomonas*
 355 *aeruginosa, Staphylococcus epidermidis, dan Micrococcus*. *Journal Of Marine Research*, Volume 1, Nomor 2, Tahun 2012, Halaman
 356 152-160.
- 357 Sulistijowati, R dan Mie, 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.
- 358 Suparmi dan Sahri, A. 2009. *Kajian Pemanfaatan Sumber Daya Rumput Laut dari Aspek Industri Dan Kesehatan*. Jurnal: Sultan Agung Vol XLIV No.
 359 96-18. Fakultas Kedokteran Universitas Islam Sultan Agung.
- 360 White, T.J., Bruns, T., Lee, S., and Taylor JW. 1990. *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. Pp. 315-322. In:
 361 *PCR Protocols: A guide to Methods and Applications*. Academic Press, Inc., New York.
- 362 Yahya, Nursyam, H., Risjani, Y., dan Soemarno. 2014. *Karakteristik Bakteri di Perairan Mangrove Pesisir Kraton Pasuruan*. Jurnal Ilmu Kelautan Maret
 363 2014 Vol. 19(1):35-42. Pascasarjana 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

The screenshot shows the OJS (Open Journal Systems) author dashboard for submission ID 6910. The top navigation bar includes links for Jurnal Kelautan, gmail - Search, [biodiv] Editor, Gmail - [biodiv], whatsapp - S, WhatsApp, NIKEN DHARMIYANTI, Menu Admin, English, View Site, and niken.dharmayanti. The main content area displays the title "Dharmayanti et al. / Antibacterial potential of symbiont bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters". Below the title, there are tabs for Workflow (selected), Publication, Submission, Review, Copyediting, and Production. Under Workflow, sub-tabs include Round 1 (selected), Round 2, and Round 3. A box titled "Round 1 Status" contains the message: "The submission must be resubmitted for another review round." A "Notifications" section lists four entries from "[biodiv] Editor Decision" with dates: 2020-11-09 04:03 PM, 2020-12-11 02:39 PM, 2020-12-30 09:52 AM, and 2020-12-30 01:35 PM. The Windows taskbar at the bottom shows various pinned icons and the date 08/02/2023.

Review 2

This screenshot is identical to the one above, showing the OJS author dashboard for submission ID 6910. The "Round 2" tab is now selected under the Workflow section. The "Round 2 Status" box displays the message: "The submission must be resubmitted for another review round." The "Notifications" section remains the same, listing four entries from "[biodiv] Editor Decision" with the same dates as the first screenshot. The Windows taskbar at the bottom shows various pinned icons and the date 08/02/2023.

Dear Editor-in-Chief,

I herewith enclosed a research article,

Title:

Antibacterial 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 Technical 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: 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

Antibacterial 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, Sujilivani, Arpan Nasri Siregar, Randi Bokhi Salampessy, Sujilivani, Siti Zachro Nurbani, Heni Budi Purnamasari, Arma 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* was *Lactobacillus plantarum*.

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

1. 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 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 (Burgesset et al., 1999; Armstrong et al., 2001; Yanet et al., 2003 in Nofiani, 2005 (Emer S and Nisreen 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 Brown algae and other types have been extensively analyzed for their antibacterial and antifungal activity (Bhakuni
34 and Rawat, 2005). Previous research on brown algae *Sargassum* sp. Has been shown to have antimicrobial potential of
35 bioactive proteins from bacteria symbiotic with it (Sartika et al. 2014) and the potential of bacterial algaeHere we evaluate
36 the properties of the brown algae *Tubunaria conoides* in producing bioactive compounds in inhibitingincluding the
37 inhibition of pathogenic bacteria Urinary Tract Infection (UTI)human pathogens (Kalaivani et al., 2016). *T. conoides* is a
38 tropical marine alga widely distributed in coastal waters in SE Asia. -We chose this alga following extensive trials on other
39 common macroalgae including *Sargassum* spp. and *Euchema cottoni*.

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

44 MATERIALS AND METHODS

45 Materials

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

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

56 MethodsProcedures

57 Sampling

58 Samples of *Tubunaria* sp. (about 1 kg wet weight) was were taken from Lima island (S: -6.001051E: E:
59 106.153804) around 1 kg for determination in the morning around 7 at low tide allows the position of algae 1 meter below
60 the water on the sidelines of the reef directly dried in the bundle of the island. Sampling was continued at 16 o'clock when
61 the low tide and taked 500 grams for antibacterial test and kept in the plastic pouch and immediately filled with seawater,
62 with seaweed : water ratio of 1: 2 until submerged, when it already arrived on Serang filled with oxygen 1 : 2 more air. The
63 seaweed stayed in the plastic with oxygen from Serang until Jakarta for a night and started done in the laboratorium in the
64 morning.Samples were maintained in fresh seawater for laboratory analyses within 24 hour of collection.

65 Identification and Determination of Macroalga

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

71 Isolation of Symbiont Bacteria Producing Antibacterial Compounds

72 Surface of Algae: Epibionts were extracted from 15 grams of algae by rinsed rinsin with 30 mL of sterile sea water.
73 The rinse water was put intoincubated in 30 mL of nutrient broth mediumthen shaken by shaker at room temperature for
74 24 hours. Inside of algae: as many asBioactive compound 15 grams of algae were rinsed with 30 mL of sterile sea water,were
75 extracted by crushed crushing 15 g of algafinely using mortalwith a mortar and pestle with the addition of 15 ml of sterile
76 seawater. The suspension is then fedwas incubated into with 30 mL broth nutrient medium and shaken by shaker aat room
77 temperature for 24 hours.

78 After extraction process, The refreshed samples of-in the 30 ml broth nutrient medium were diluted into 9 ml broth
79 nutrient sterile by-10¹ up to 10⁻⁵. Each dilutions was were grown on a plate count agar medium by incubate them at 37 °C
80 for 2 x 24 hours. After incubating the petri dishes which were contain samples from each dilution, then the colonies bacteria
81 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 *Tubunaria 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 them on
87 slant agar medium, with a clear code.

88 Selection of Symbiont Bacteria Isolates Antagonistically against Pathogenic Bacteria

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

93 Inhibition zones were read as the point determined as those showing clear zones around the colony of symbiont
94 bacteria isolates, the more clear zone of isolates inhibit for both *Escherichia coli* and *Staphylococcus aureus*, are the better
95 their activity. Strains that showed maximum antagonistic effect against tested pathogens were choosed and marked by its
96 eodeidentified. Isolates that These chosen isolate with appropriate code which was formed a clear zone or has with the a
97 highest activity are waswere isolated and selected for further antibacterial testing by paper disc and identification of
98 phenotype and genotype, testing.

99 Antibacterial Potential Testing of Symbiont Bacterial Isolate by Paper Disc Diffusion

100 Testing inhibitory the supernatant of symbiont bacteria on thefor inhibitory growth of *E.coli* and *S.aureus* was
101 performed by the agar diffusion method (Hudzieki, 2009Grela E et al. 2018) REFERENCE. Supernatant was obtained by
102 separating the filtrate and supernatant by centrifugation processcentrifuge for 1 hour, temperature at (25 °C and 3000 rpm).
103 Paper discs containing supernatant 40 µL and the negative control nutrient broth 40 µL which has allowed were dried left
104 for 1 hour to reduce the water excess (dried), negative control nutrient broth 40 µL also has allowed for 1 hour to reduce
105 the water excess (dried), and positive control chloramphenicol 0.01 mg/mL, were placed on the surface of the Mueller Hinton
106 Agar A medium containing 1 mL test bacteria. Furthermore and incubated for 2 x 2448 hours at 37 °C. The supernatant
107 diffuses from the disc into the agar in decreasing amounts the further it is away from the disc. If the organism is killed or
108 inhibited by both the supernatant and chloramphenicol as antibiotic positive control, there will be no growth in the immediate
109 area around the disc, this is called the zone of inhibition. The zone sizes were compared up on a standardized to give a
110 result of to assess bioactivity as sensitive, resistant, or intermediate, te then It was observed and measured its in each case
111 the resistance zone where shows no colonies growth with by a ruler was measured by using ruler to the nearest mm.

112 Identification of Phenotype and Genotype of Symbiont Bacteria

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

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

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

137 Formatted: Font: 10 pt

138 Formatted

139 Formatted: Space After: 6 pt

140 Formatted

141 Commented [aa5]:

142 Formatted

143 Commented [p6]: This needs to be clarified.

144 Formatted: Font: 10 pt

145 Formatted: Space After: 6 pt

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

148 Formatted: Font: 10 pt

149 Formatted

150 Commented [N08]:

151 Formatted: Font: 10 pt

152 Commented [aa9]:

153 Formatted

154 Commented [p10]: Spell out MHA

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

156 Formatted: Font: 10 pt

157 Commented [aa12]:

158 Commented [aa13]: The meaing of resistance zone

159 Commented [N014]:

160 Commented [aa15]:

161 Commented [N016]:

162 Formatted: Font: 10 pt

163 Formatted

164 Formatted: Font: 10 pt

165 Formatted: Font: 10 pt, English (Australia)

166 Formatted: Font: 10 pt

167 Formatted: Space After: 0 pt

168 Formatted

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

171 Formatted

RESULTS RESULTS AND DISCUSSION

3.

The Result of Identification and Determination of Macroalgae

The macroalgae observation area and the sample site obtained are determined based on the location coordinate point. The location of macroalgae-observation S: 6.001051; E: 106.153804 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 s 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.

Tabel 21. Macroscopic forms of bacterial colonies

No	Colony code	Shape	Color	Edges	Morphology of colonies

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

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 (¹), (²), (³), (⁴) 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 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 32. 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

Observations of bacteria can be done individually or in groups in the form of colonies. If the 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 RSwidjoseputro, 1981-2012).

The Selection Results Symbiont Bacteria Producing Antibacterial Compounds

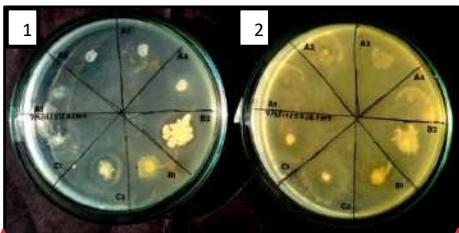


Figure 12. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

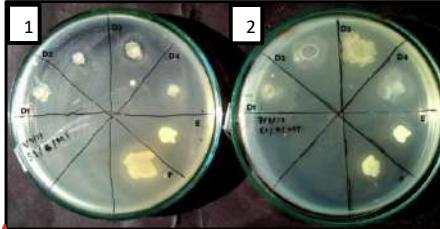


Figure 23. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

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* 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 10⁴ 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 10⁴ dilution, the second colony of the isolated third plate, and a colony obtained in the second repetition.

Bacterial isolates derived from the insidetissue showed have better activitybetter inhibition than bacterial isolates derived from the surfaceepibionts. 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,Jay, 49942016), 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.

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 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

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

247
248 Positive control:
249 inhibition against *S. aureus* and *E. coli* on a paper disc is
250 of chloramphenicol.
251 sensitive to positive
252 the absence of active
253 medium has no effect.



254 of test bacteria with 16.8 mm
255 col with a concentration of 0.03
256 nm (Lay, 1994), while the dose
257 can be said that bacteria Test is
258 bacterial inoculation) indicates
259 at a supernatant still containing

260 Commented [p22]: This is Discussion

261 Formatted: Font: 10 pt

262 Formatted: Font: 10 pt

263 Commented [p23]: This is also discussion

264 Formatted: Font: 10 pt

265 Formatted: Font: 10 pt

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

268 Formatted: Font: Italic

269 Formatted: Font: Italic

270 Formatted: English (Australia)

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

272 From the stability of the measured inhibition zone, in general the antibacterial properties of the supernatant
273 produced by the symbiotic bacteria act as bactericidal against Gram positive bacteria and are merely bacteriostatic in Gram
274 negative. Paper disc with a supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a
275 48 hour incubation period. While against Gram negative bacteria, around the disc paper shows the presence of inhibitory
276 activity but gradually become turbid before the incubation period reaches 24 hours. Antimicrobial agents may be
277 bacteriostatic at low concentrations but are bactericidal at high concentrations (Fernando B and Bruce RLLay, 19942020).
278 Other factors that influence the ability of inhibitory inhibition are the concentration or intensity of antimicrobial agents, the
279 number of microorganisms, the temperature, the species of microorganisms, the presence of organic matter and the degree
280 of acidity (pH) (Manisha DM and Shyamapada M, 2011Sulistijowati and Mile, 2015).

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

Repetition	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

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

288 Formatted: Font: 9 pt

289 Formatted: Font: 10 pt

290 Formatted: Font: 10 pt

291 Formatted: Font color: Text 1

292 Formatted: Font color: Text 1

293 Formatted: Font color: Text 1

294 Formatted: Font: 9 pt

295 Formatted Table

296 Formatted: Font: 9 pt

297 Formatted: Font: 9 pt

298 Formatted: Font: 10 pt

299 Formatted: Font: 10 pt

300 Formatted: Font: 10 pt, Italic

301 Formatted: Font: 10 pt

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 In fact, marine bacteria are significant reservoirs of a plethora of bioactive molecules which have never been found in terrestrial organisms. (Giovanna R, 2020 Gudbjarnason 1999 in Nofiani, 2005). **-Seawater contains an active inhibitor agent for Gram positive bacteria;** according to (Garima K et al. Okami (1982)2) in Nofiani (20052017) that seawater contains an active inhibitor agent for organisms, seawater has the ability of inhibitors a against Gram positive bacteria .

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

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).

Identification of Phenotype and Genotype of Symbiont Bacteria

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*.

Based on phenotypic identification results through cell staining and biochemical testing, symbiont bacteria have been rod shaped, non acidic, non spore forming, non motile, aerobically grown, negative catalase, and positive to carbohydrate tests. In general, the identification of microscopically selected isolates showed specific characteristics possessed by lactic acid bacteria (*Lactobacillus spp.*), such as round colonies, milky white, Gram positive with short stem cells, and does not form endospores (Desnair 2012 in Saskia, Davoodabadi et al. 2014/2015). The genus *Lactobacillus* can be isolated from several different habitats, e.g. from milk/fish intestine (Sulistijowati and Mile, 2015), bekasam products (Ingratuban et al., 2013), up to coastal mangrove waters (Yahya et al., 2014).

The DNA of the symbiont 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 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*.

Lactobacillus plantarum 100%

GCTCAGGACGAACGCTGGCGCTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGGTGTGCTTCATGATTACAT
TTGAGTGGACTGGCAGACTGGTAGAACACGCTGGGAAACCTGCCAGAACGGGGGATAACACCTGAAACAGATGCTAACTACCG
CATAACAACTTGGGACCGCATGGCGACTGGAGGAAATGGCTTCGGCTTACCTTTGGTAGGTCGGCGCTTAGGCTAGATG
TGCGGGGAAACGGCTCACATGGCAATGACAGTACCCGCCAGGGTAACTGGGACACTGGGACTAGAACGCCAAA
CTCCCTACGGGAGGAGCAGTAGGGAACTTCCCAAACTGGAGGAAACAGTCTGGTAGGACGCCGCTGAGTGAAGGAGGTTT
GGCTCGTAAACTCTGTTTAAAGAACATATCTGGAGAACACTGTTGGTAGGTTTACCGGTTTAAACCAAAGGCCACGGCTA
ACTACGTCCAGCAGCGGGTAACTCGTAGGTGGCAAGCGTTGGATTATGGCGTAAGCGAGCGCAGCGGGTTTT
AAGCTGTAGTGAAGACCTTCCGGCTAACCGAAGAAGTCTGGCAAGAACACTGGAAACTGGTAGTCGAAGAGGACCTGGAACTC
CATGTTGAGCGGTAAATGGCTAGATATTGGAAAGAACACCATGGCGAACGGCGCTGTCTGGTAGTAACTGACCGTGGCTC
GAAAGTATGGTAGAACACAGGATTAGTACCCCTGGTAGTCCATACCGTAACCGATGAATGCTAAGTGTGGAGGGTTCCGCC
TCAGTCTGGCAGCTAACGCTTACCGGCTGGGAGTACGGCCGCAAGGGCTGAACAACTAACAGGAAATTGACGGGGGCC
CACAAAGGGTGGAGCTGGTTAACCTGGCAAGAACACTTACCGAGGTCTGGACATACTGCAAACTCAAGAGATT
GAGCTTCCCTGGGGACATGGATACAGGGTGTGATGGTGTCTGAGCTGGTAGTGGGTAAGTCCCGCAACG
AGCGAACCTTATTCAGTGGCAGCTTAAGTGGGCAACTCTGGTAGACCTGGCGTGAACACGGAGGAAGTGGGAGT
ACGTCATACATGCCCCCTTATGACTGGGCTACACAGCTGGCTAACATGGTAGTACACCGTAGTGGCAACTGCCGAGAGTAAG
ATGCTCTTAAAGGACCTTCTAGTGGGAGTGGTAGGCTGCAACTGCCCTACATGAGCTGGGAATCTGGTAGTAACTGCCGATCAGC
ATGGCGGGTAAACTGTTCCGGGCGCTTGTACACAGGCCGGTACACCATGAGATGGTAGTGGTAACACCCAAAGTC

Figure 4. Sequens of 16S rDNA

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

4. CONCLUSION

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

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

ACKNOWLEDGEMENTS

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

REFERENCES

- 351 Alessandro B, Christine AM, Brendan FG. 2017. Marine macroalgae and their associated microbiomes as a source of antimicrobial chemical diversity. *Eur J. of Phycol.* 52(4): 452-465.
352 Andreu 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
353 of prebiotic and antioxidant value. *Antioxid (Basel)*, 2019 Sep; 8(9): 406.
354 Arumugam P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant and anticancer properties of *Turbinaria conoides* (J. Agardh). *Clin Phytosci*. (2017) 3:5.
355 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
356 seaweeds: looking at chemical composition, phytopharmacology and cosmetic applications. *Mol*, 2019 Nov; 24(22): 4182.
357 Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D; Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015. Antibacterial
358 activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Pubmed.
359 Emer Shannon, and Nisreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological mechanisms and
360 Applications. *Mar Drugs*. (2016) Apr; 14(4): 81.
361 Erin R sanders, 2012. Aseptic Laboratory Techniques: Plating Methods. *J Vis Exp*. 2012; (63): 3064.
362 Fernando Baquero and Bruce R. Levin , 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. *Nat Rev Microbiol*. (2020)
363 Garima Kapoor, Saurabh Saigal, and Ashok Elongavan, 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. *J Anaesthesiol Clin Pharmacol*. 2017 Jul-Sep; 33(3): 300–305.
364 Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. res* (2016).
365 Grela E, Kozłowska J, Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem*;120(4):303–311.
366 Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol*. 2011;22:315–26.
367 Irma Esthela Soria-Mercado, Luis Jesús Villarreal-Gómez, Graciela Guerra Rivas and Nahara E, Ayala Sánchez, 2011. Bioactive Compounds from Bacteria
368 Associated to Marine in Algae Biotechnology: Molecular Studies and Novel Applications for Improved Quality of Human L. BoD – Books on Demand, 2012 (252).
369 Kalaiyani G, Hemalatha, N, dan Poongothai. 2016. Screening Of Marinene Brown Algae Associated Potential Bacteria Producing Antagonistic
370 Bioactive Compounds Against Utii Pathogens. *International Journal of Pharma and Bio Sci*, 2016 April; 7(2): (B) 395 – 405. India.
371 Manisha DM, and Shyamapada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed*. 2011 Apr; 1(2): 154–160.
372 Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs*. 2016 Mar; 14(3): 52.
373 Mark LW, Philippe P, James SC, John AR, Sabeeha SM, Katherine EH, Alison GS, Mary EC, Susan HB, 2016. Algae as nutritional and functional food
374 sources: revisiting our understanding. *J of Appl. Phycol.* volume 29 pages 949–982 (2017).
375 Mounvr B, Moulay S, and Saad KI, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. *J Pharm Anal*. 2016 Apr; 6(2): 71–79.
376 Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for Pharmacology. *Mar
377 Drugs*, 2019 Aug; 17(8): 464.
378 Phumudzo T, Ronald N, Khayalethu N, Phatuwani M, 2013. Bacterial species identification getting easier . *Afr J. of Biotechnol*, Vol. 12(41), pp. 5975-
379 5982.
380 White, Bruns T, Lee S, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322. In PCR
381 Protocols: A guide to Methods and Applications, Academic Press, Inc., New York.
382 Bhakuni, D.S dan Rawat, D.S. 2005. *Bioactive Marine Natural Products*. ISBN 1-4020-3472-5 (HB). Published by Springer New York 10013, USA
383 and Anamaya Publishers, New Delhi, India.
384 Cowan, S.T and Steel, K.J. 1993. *Manual for the Identification of Medical Bacteria*. 3rd Edition. University of Cambridge, UK.
385 Dwidjosepuro, D. 1981. *Dasar-dasar Mikrobiologi*. Cetakan ke 5. Djambatan, 1981; Jakarta.

Formatted

Formatted

Commented [p28]:

Commented [p29]: This is just repeating the results. Can be

Formatted

- 392 Hudzicki. 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)
- 393 Ingratubun, J. A., Ijung, F. C., dan Oimbala, 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.
- 394 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.
- 395 Kusumadewi, R. 2004. *Penapisan Awal Senyawa Bioaktif Antibakteri dari Melati Laut (Clerodendrum inerme)*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor: Bogor.
- 396 Lay, B. W. 1994. *Analisis Mikroba di Laboratorium*. PT. Rajawali Persada: Jakarta.
- 397 Lakman, J.B., Dwiyana 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.
- 398 Nofiani, R. 2005. *Urgensi dan Mekanisme Biosintesis Metabolit Sekunder Mikroba Laut*. Jurnal Natur Indonesia 10 (2), April 2008: 120-125.
- 399 O'Donnell K. 1993. *Fusarium and its near relatives*. In: Reynolds DR & Taylor JW (Eds). *The Fungal Holomorph: Mitotic, Meiotic and Pleiomorphic Speciation in Fungal Systematics* (pp 225–233). CAB International, Wallingford, UK.
- 400 Pitcher DG, Saunders NA, Owen RJ. 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Letters in Applied Microbiology 1989; 8: 151-156.
- 401 Pelezar, M.J dan Chan, E.C.S. 1986. *Dasar dasar Mikrobiologi*. Diterjemahkan oleh Ratnasari, dkk. Edisi 1. UI Press, Jakarta.
- 402 Sahara, F. N. I., Radjasa, O., K. dan Supriyatini, E. 2013. *Identifikasi Pigmen Karotenoid pada Bakteri Simbion Rumpun Laut Kappaphycus 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>.
- 403 Sartika, Ahmad, A., dan Natsir, H. 2014. *Potensi Antimikroba Protein Bioaktif dari Bakteri Simbion Alga Coklat Sargassum sp. Asal Perairan Pulau Lao-lao*. Jurnal: FMIPA Universitas Hasanuddin. Makassar.
- 404 Saskia, A. 2014. *Pengembangan Kultur Kering Bakteri Lactobacillus plantarum (SK5) asal Bekasan sebagai Kandidat Probiotik dengan Teknik Pengeringan Buku*. Skripsi. Fakultas Perikanan dan Ilmu Kelautan, Institut Pertanian Bogor: Bogor.
- 405 Siregar, A. F., Sabdono, A., dan Pringgenies, D. 2012. *Potensi Antibakteri Ekstrak Rumpun Laut Terhadap Bakteri Penyakit Kulit Pseudomonas aeruginosa, Staphylococcus epidermidis, dan Mierococcus*. Journal Of Marine Research. Volume 1, Nomor 2, Tahun 2012, Halaman 152-160.
- 406 Sulistijowati, R. dan 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.
- 407 Suparmi dan Sahri, A. 2009. *Kajian Pemanfaatan Sumber Daya Rumpul Laut dari Aspek Industri Dan Kesehatan*. Jurnal. Sultan Agung Vol XIIV No. 96: 18. Fakultas Kedokteran Universitas Islam Sultan Agung.
- 408 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.
- 409 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. Pascasarjana 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

Copy Editing

The screenshot shows the OJS interface for a manuscript submission. The top navigation bar includes links for Jurnal Kelautan, gmail - Search, [biodiv] Editor, Gmail - [biodiv], whatsapp, WhatsApp, NIKEN DHARI, Menu Admin, English, View Site, and nikendharmayanti. The main title of the manuscript is "Antibacterial potential of symbiont bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters". The workflow tabs are Workflow and Publication, with Copyediting selected. The Copyediting tab displays a section for "Copyediting Discussions" with no items listed. The Copiedited tab shows a file named "34804-2_editors_D220145-Turbinaria conoides - Darmayanti+.doc (2)" uploaded on December 31, 2020. The status bar at the bottom indicates the URL <https://smujo.id/biodiv/55call55/tab/author-dashboard/author-dashboard-tab?submissionId=6910&stageId=4>, a search bar, and system status like "Hujan sore hari" and "9:31 08/02/2023".

Production

The screenshot shows the OJS interface for the same manuscript, now in the Production stage. The status is listed as "Published". A red banner at the top states "This version has been published and can not be edited." The manuscript details are as follows:

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

Abstract: Darmayanti N, Anti A, Siregar RR, Sipohutar Y, Permodi A, Siregar AN, Salampessy RB, Syulyanti, Nurbani 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 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 molecular analysis was performed using Next-Generation Sequencing (NGS) to determine the bacterial species.

The status bar at the bottom indicates the URL <https://smujo.id/biodiv/55call55/tab/author-dashboard/author-dashboard-tab?submissionId=6910&stageId=4>, a search bar, and system status like "27°C Kabut" and "9:31 08/02/2023".

1 Antibacterial potential of symbiont 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*, *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-symbiont 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 *T*. 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 Nisreen AG 2016). *T*he form of symbiotic mutualism. Algae provide needed
33 sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiont bacteria isolates in
34 algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a results 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 cottoni*.

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 symbiont 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 alga with 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 at 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 (*Escherichia 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 *Escherichia 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
70 °C. 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 isolate^[isolate] 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 cycles}
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 insert a reference paper for this procedure???

Commented [A2]: Something is missing here

101 **Tabel 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 alga

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

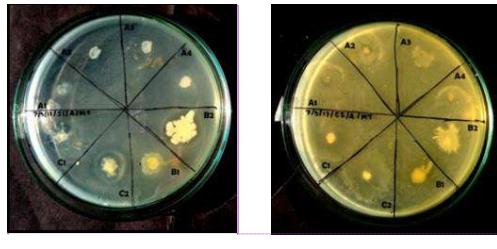
106 number code representing isolates in the order of the first, second, and so on according the best inhibition zone of each colony observed

107 on the plate

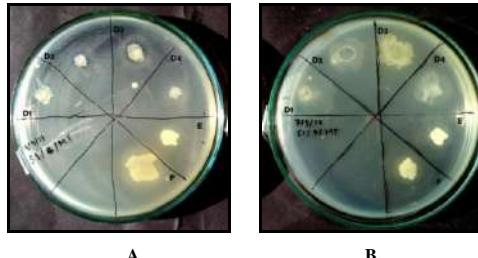
108 **** The code of number 2 identifies the isolate obtained from the second repeat

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 Symbiont Bacteria Producing Antibacterial Compounds**113 **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)

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

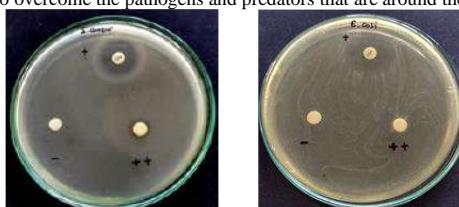


114 **Figure 2.** Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

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 (Mouny 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

The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Mounyr Balouri et al, 2016. a measured inhibition zone of less than 10 mm shows weak activity 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 chloramphenicol control. This is because the antibacterial compound of the extracted symbiont bacteria was a 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. In fact, marine bacteria are significant reservoirs of a plethora of bioactive molecules which 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)

Identification of Phenotype and Genotype of Symbiont Bacteria

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

Lactobacillus plantarum_100%

```
GCTCAGGACGAACGCTGGCGCGTGCCTAATCATGCAGTCGAACGAACTCTGGATTGGTGCCTGCATCATGATTTC
CATTTGAGTGAGTGGCAACTGGTAGTGAGAACAGCTGCCAGAGGGATAACACCTGAAACAGATGCTAATA
CCGCATAACAACCTGGGACCGCATGGTCCGAGCTTGAAGATGGCTTCGGCTATCACTTTGGATGGTCCCGCCGGTATTAGCTAG
ATGGTGGGGTAACCGCTCACCATGGCAATGAGTGGCCACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACCGCCC
AAACTCCTACGGGAGGGCAGCAGTAGGGAACTTCCACAATGGCAAGAACAGTGTGGATGGAGCAACCCGGTGAAGTGAAGAAGGGTT
TCGGCTCGAAAAACTCTGTTAAAGAAGAACATATCTGAGAGTAACCTGTCAGGTATTGACGGTATTAACAGAAAGCCACGGC
TAACCTGTCAGCGAGCCGGTAAACTCGTAGGTGGCTCGGATTTATGGGCGTAAAGCGAGCCGAGGGGTTTT
TAACTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAACGTCATCGGAAACTCTGAGTCAGAACAGGACAGTGCAC
TCCATGTTAGCGGTGAATGCGTAGATATGGAAAGAACACCGACTGGCGAAGGGGCTGCTGCTGCTGTAACGAGCTGAGGC
TCGAAGGTATGGGTAGCAACAGGATTAGATACCCCTGGTAGTCATACCGTAAACGATGAATGCTAAGTGTGGAGGGGTTCCGCC
CTTCAGTGTGCACTAACGCATTAAGCATTCCGGCTGGGGAGTAGCGGCCAGGCTGAACACTCAAAGGAATTGACGGGGGCC
GCACAAAGCGCTGGAGCATGCTGGTTAAATTCGAAGGCTACGGCAAGGAACCTTACCGAGCTTGAACATCTGCAAAATCTAAGAGATT
AGACGCTCCCTCCGGGACATGGATACAGGTGTGTCAGCTGGTGTCTGGATGTGGTTAAGTCCCGAAC
GAGCGCAACCCATTATTCAGTTGCCAGCATTAAGTTGGGCACTCTGGTAGACTGCCGGTGACAAACCGGAGGAAGGTGGGAT
GACCGTCAAATCATCATGCCCTTATGACCTGGGCTACACAGTGGTACAAATGGTGTGACAGTTGCGAAGCTCGCAGAGTAA
GCTAATCTTAAAGGCATTCTCAGITCGGATTTAGGCTGCAACTCGCCATCATGAAGTGGAAATCGCTAGTAATGCGGATCAG
CATGCCGGGTGAATACGTTCCGGGCTTGTACACACCAGGCGTACACCATGAGAGTTGTAACACCCAAAGTC
```

Figure 4. Sequens of 16S rDNA

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

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

ACKNOWLEDGEMENTS

This paper and the research behind it 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 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. Agardhi). *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.
Microbiol.* (2020)
- 188 Garima Kapoor, Saurabh Saigal, and Ashok Elongavan, 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. *J
Anaesthesiol Clin Pharmacol.* 2017 Jul-Sep; 33(3): 300–305.
- 189 Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. res*
190 (2016)
- 191 Grela E., Kozlowska J., Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem.*;120(4):303–
192 311
- 193 Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol.*
194 2011;22:315–26.
- 195 Irma Esthela Soria-Mercado, Luis Jesús Villarreal-Gómez, Graciela Guerra Rivas and Nahara E. Ayala Sánchez, 2011. Bioactive
196 Compounds from Bacteria Associated to Marine in Algae.Biotechnology: Molecular Studies and Novel Applications for Improved
197 Quality of Human L. BoD – Books on Demand, 2012 (252)
- 198 Kalaivani , G., Hemalatha , N., dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria Producing
Antagonistic Bioactive Compounds Against Ut Pathogens.* International Journal of Pharma and Bio Sci. 2016 April; 7(2): (B) 395 –
199 405. India.
- 200 Manishi DM and Shyamapada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed.* 2011 Apr;
201 1(2): 154–160.
- 202 Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs.* 2016 Mar; 14(3): 52.
- 203 Mark LW, Philippe P, James SC, John AR, Sabeeha SM, Katherine EH, Alison GS, Mary EC, Susan HB, 2016. Algae as nutritional and
204 functional food sources: revisiting our understanding. *J. of Appl. Phycol.* volume 29 pages 949–982 (2017)
- 205 Mounyr B, Moulay S and Saad KI, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal.* 2016 Apr;
206 6(2): 71–79.
- 207 Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for
208 Pharmacology. *Mar Drugs.* 2019 Aug; 17(8): 464.
- 209 Phumudzo T, Ronald N, Khayalethu N, Fhatuwani M, 2013. Bacterial species identification getting easier . *Afr J. of Biotechnol.* Vol.
210 12(41), pp. 5975-5982
- 211 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp.
212 315-322. In PCR Protocols: A guide to Methods and Applications, Academic Press, Inc., New York.
- 213
- 214
- 215

1 Antibacterial potential of symbiont 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*, *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-symbiont 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 *T*. 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 Nisreen AG 2016). *T*he form of symbiotic mutualism. Algae provide needed
33 sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiont bacteria isolates in
34 algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a results 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 symbiont 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 alga with 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 at 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 (*Escherichia 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 *Escherichia 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
70 °C. 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 isolate^[isolate] 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 cycles}
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 insert a reference paper for this procedure???

Commented [A2]: Something is missing here

101 **Tabel 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 alga

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

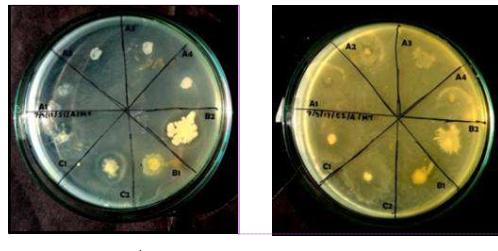
106 number code representing isolates in the order of the first, second, and so on according the best inhibition zone of each colony observed

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 Symbiont Bacteria Producing Antibacterial Compounds**113 **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)

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

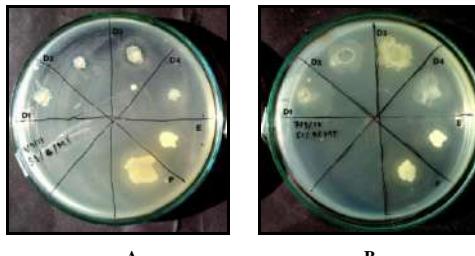


Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

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 (Mounyr B et al., 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.

The antibacterial compounds produced by symbiont bacteria isolates showed different inhibitory activity against both tested bacteria *S.aureus* and *E.coli*. According to Irma ESM (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 less suspected because it requires higher defense power to overcome the pathogens and predators that are around the algae.

Formatted: Font: Italic

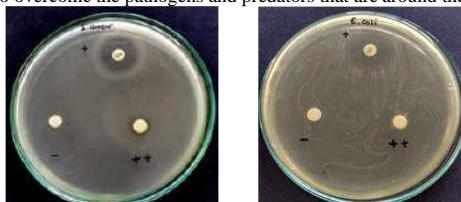


Figure 3. Results of antibiotic susceptibility test against *S. aureus* and *E. coli*

Antimicrobial agents may be bacteriostatic at low concentrations but are bactericidal at high concentrations (Fernando B and Bruce RL, 2020). Other factors that influence 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) (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

	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 Balouri 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~~sing~~, 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%

```
GCTCAGGACGAACGCTGGCGCGTGCCTAATCATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCCTGCATCATGATTAA
CATTTGAGTGAGTGGCGAACCTGGTAGACTAACAGTGGGAAACCTGCCAGAGCGGGGATAACACCTGGAACAGATGCTAATA
CCGCATAACAACACTGGGACCGCATGGTCCGAGCTTGAAGAGATGGCTTCGGCTATCACTTTGGATGGTCCCGCCGGTATTAGCTAG
ATGGTGGGGTAACCGCTCACCATGGCAATGAGTGGCCACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACCGGCC
AAACTCCTACGGGAGGGCAGCAGTAGGGAACTCTTCCAATGGGAAAGCTGATGGTCCGAGACAGCTGGCGTGAAGTGAAGAAGGGT
TCGGCTCGAAAAACTCTGTTAAAGAAGAACATATCTGAGAGTAACCTGTCAGGTATTGACGGTATTAAACAGAAAGGCCACGGC
TAACCTGTCAGGCGAGCAGCGGTTAACGGCTAGGTTAACGGCTAGGTTAACGGCTAGGTTAACGGCTAGGTTAACGGCTAGGTT
TAACTCTGATGTAAGGCTTCGGCTCAACCGAAAGTGCATCGGAAACTCTGAGTGCAGAACAGGACAGTGCAC
TCCATGTTAGCGGTGAATGGCTAGATATGGAAAGAACACCGACTGGCGAAGGGGCTGCTGCTGCTGTAACCTGACGCTGAGGC
TCGAAGGTATGGGTAGCAACAGGATTAGATACCCCTGGTAGTCCATACCGTAACAGTGAATGCTAAGTGTGGAGGGTTTCCGCC
CTTCAGTGTGCACTAACGCATTAAGCATTCCGGCTGGGGAGTACGGCCCAAGGCTGAACACTCAAAGGAATTGACGGGGGCC
GCACAAAGCGCTGGAGCATGGTTAACGGCTAACGGCAAGGAACCTTACCGAGCTGGACATATACTGCAAAATCTAAGAGATT
AGACGCTCCCTCCGGGACATGGATACAGGTGTGTCAGGTTGCTCAGGCTGTCTGTGAGATGTTGGTTAAGTCCCGAAC
GAGCGCAACCCATTATTCAGTTGCCAGCATTAAGTTGGGCACTCTGGTAGACTGCCGGTGACAACACGGAGGAAGGTGGGAT
GACCGTCAAATCATCAGTGGCTACACAGCTGGTACAAATGGATGGTACAACTGGAGTTGCGAAGCTCGCAGAGTAA
GCTAATCTTAAAGGCATTCTCAGITTCGGATTTAGGCTGCAACTGCCATCATGAAGTGGAAATCGCTAGTAAATGCCGGATCAG
CATGCCGGGTAAACGTTCCGGGCTTGTACACACCGCCGTACACCATGAGAGTTGTAACACCCAAAGTC
```

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. Agardhi). *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.
Microbiol.* (2020)
- 188 Garima Kapoor, Saurabh Saigal, and Ashok Elongavan, 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. *J
Anaesthesiol Clin Pharmacol.* 2017 Jul-Sep; 33(3): 300–305.
- 189 Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. res*
190 (2016)
- 191 Grela E., Kozlowska J., Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem.*;120(4):303–
192 311
- 193 Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol.*
194 2011;22:315–26.
- 195 Irma Esthela Soria-Mercado, Luis Jesús Villarreal-Gómez, Graciela Guerra Rivas and Nahara E. Ayala Sánchez, 2011. Bioactive
196 Compounds from Bacteria Associated to Marine in Algae.Biotechnology: Molecular Studies and Novel Applications for Improved
197 Quality of Human L. BoD – Books on Demand, 2012 (252)
- 198 Kalaivani , G., Hemalatha , N., dan Poongothai. 2016. *Screening Of Marinene Brown Algae Associated Potential Bacteria Producing
Antagonistic Bioactive Compounds Against Ut Pathogens.* International Journal of Pharma and Bio Sci. 2016 April; 7(2): (B) 395 –
199 405. India.
- 200 Manishi DM and Shyamapada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed.* 2011 Apr;
201 1(2): 154–160.
- 202 Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs.* 2016 Mar; 14(3): 52.
- 203 Mark LW, Philippe P, James SC, John AR, Sabeeha SM, Katherine EH, Alison GS, Mary EC, Susan HB, 2016. Algae as nutritional and
204 functional food sources: revisiting our understanding. *J. of Appl. Phycol.* volume 29 pages 949–982 (2017)
- 205 Mounyr B, Moulay S and Saad KI, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal.* 2016 Apr;
206 6(2): 71–79.
- 207 Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for
208 Pharmacology. *Mar Drugs.* 2019 Aug; 17(8): 464.
- 209 Phumudzo T, Ronald N, Khayalethu N, Fhatuwani M, 2013. Bacterial species identification getting easier . *Afr J. of Biotechnol.* Vol.
210 12(41), pp. 5975-5982
- 211 White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp.
212 315-322. In PCR Protocols: A guide to Methods and Applications, Academic Press, Inc., New York.
- 213
- 214
- 215

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

3
4
5
6
7 **Abstract.** Brown seaweeds have the potential to produce bioactive compounds. It has been shown that the bacteria-Bacteria associated
8 with 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 found bacteria were isolated, 6 of which came 6 were isolated from external
11 tissue, while 8 came from internal tissue. Through the Results of antagonistic test revealed that, 7 isolates showed inhibitory activity
12 against *Staphylococcus aureus* and only 1 isolate showed the inhibition against both *S.aureus* and *E.coli*. Phenotypic and genotypic
13 identification analysis showed that the symbiont bacteria species was *Lactobacillus plantarum*.

14 **Keywords:** bioassay Bioassay, brown seaweed, 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 are particularly used in
23 the design of new antimicrobial drugs. Research for the identification of promising algal species, standardization of
24 analytical methods, isolation of compounds through integrated fractionation of bioassays, detailed chemical
25 characterization and evaluation of their safety, evaluation of synergistic effects between components, and efforts to
26 improve yields, and lowering extraction costs is needed (Marie JP et al. 2016).

27 In later decades, made strides microbiological procedures have altogether made a difference in build-up phylogenetic
28 affiliations of seaweed-related epi bacterial communities and endophytes. Be that as it may, there's inadequately
29 prove that utilitarian connections for seaweed-bacterial intuitive can be built up and well caught on. Epiphytic bacterial
30 communities are quick rapid colonizers of the ocean growth surface, some of the time versatile and able
31 to quickly metabolize algal exudates (Singh R.P and Reddy C.R.K. 2014). It's It has traditionally been used for childrens
32 fever, as a fertilizer, repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011).
33 Seaweeds can secrete secondary metabolites with antibacterial properties (Emer S and Nisreen AG-2016). The form of
34 symbiotic mutualism. Algae provide needed essential sites and nutrients, while the bacteria encourage growth and protect
35 the algal surface against symbiont bacteria isolates in as algae have abundant antimicrobial activity. The existence of the
36 bacteria is suspected as a result of infections acquired from the community (Arumugama P-et al. 2017). *T. conoides* is a
37 tropical marine alga widely distributed in coastal waters in Asia. It here we evaluate This study evaluates the properties of
38 the brown alga *Turbinaria conoides* in producing bioactive compounds including the inhibition of human pathogens
39 (Kalaivani et al. 2016). *T. conoides* is a tropical marine alga widely distributed in coastal waters in Asia. We chose this
40 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.

41 **MATERIALS AND METHODS**

42 **Procedures**

43 **Sampling**

44 Samples of *Turbinaria* sp. (about 1 kg wet weight) were taken from Lima island (S: -6.001051; E: 106.153804)
45 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 10^3 . 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.

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.

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 surface of 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 these 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.

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

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* was
69 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 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 mediumThe 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 [K6]: This reference is not found in the reference section. Check it.

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

111 **Tabel 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:

*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 the best inhibition zone of each colony observed on the plate

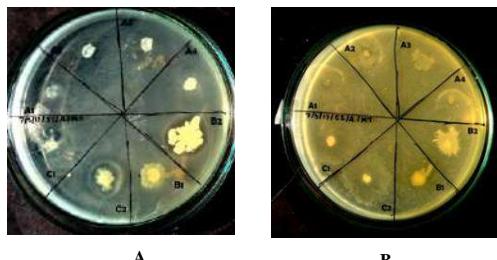
**** The code of number 2 identifies the isolate obtained from the second repeat

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

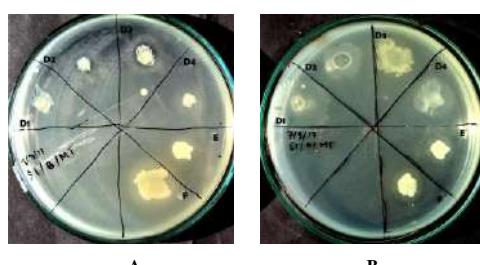
Code of isolates	Solid medium	
	Shape	Color
TUL ² -A1-2	Spread	Milky white
TUL ² -A2-2	Spread	Milky white
TUL ² -A3-2	Spread	Milky white
TUL ² -A4-2	Spread	Milky white
TUL ² -B1-2	Rhizoidal	Cloudy white
TUL ² -B2-2	Rhizoidal	Cloudy white
TUD ⁴ -C1-2	Spread	Milky white
TUD ⁴ -C2-2	Spread	Milky white
TUD 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
 121 different for each species and it is characteristic of a particular species (Erin RS 2012). Bacteria were isolated in a solid
 122 medium and the size of the colony was different for each species and was characteristic of a particular species (Erin 2012).

123 The selection results symbiont bacteria producing antibacterial compounds

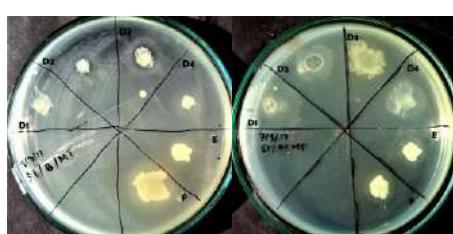


124 **Figure 1.** Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)



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 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 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 symbiont 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 Balouri et al. (2016), a measured less than 10 mm inhibition zone of less than 10 mm shows 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.

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

The Genotypic result through molecular identification is carried out 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 sequenced in impact with 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*. Greatest Highest 100% personality, greatest score 2660, add up to score 2660, 100% inquiry scope, E esteem 0, was recorded to for the taxon of adjacent microbes. [The classification of bacterial confines is as takes after: Microscopic organisms; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; *Lactobacillus*; *Lactobacillus plantarum*.]

Commented [K10]: Please rewrite this line.

Sequens of 16S rDNA

GCTCAGGACGAACGCTGGCGCGTGCCTAATACATGCAAGTCGAACGAACACTCTGGTATTGATTGGCTTGCATCATGAT
TTACATTGAGTGAGTGGCGAACACTGGTAGTAACACGGGAAACCTGCCAGAAGCGGGGATAACACCTGGAAACAG
ATGCTAATACCGCATAACAACCTGGACCCATGGTCCGAGCTGAAAGATGGCTTCCGCTATCACTTTGGATGGTCCCG
CGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCTGGCAATGATACGTAGGCCGACCTGAGAGGGTAATCGGCCACA
TTGGGACTGAGACACGGCCAACCTCCAGGGAGGCGAGCTAGGGATCTCCACATGGACGAAAGTCTGATGGAG
CAACGCCGCGTAGTGAAGAAGGGTTCCGCTCGTAAACACTCTGTTAAAGAAGAACATATCTGAGAGTAAGTGTCA
GGTATTGACGGTATTAACAGAAAGGCCACGGCTAAGTACCGTGCAGCAGCCGGTAATACGTAGGTGGCAAGCGTTG
TCCGGATTITATGGCGTAAAGCGAGGCCAGGGCGTTTTAAAGTCTGATGTGAAAGCCTCGGCTCAACCGAAGAAGTG
CATCGGAAACTGGGAAACTTGTAGTGAGCAGGAGCAGCTGGAAACTCCATGTTAGCGGTGAAATCGGTAGATATATGGA
AGAACACCGTGGCGAAGGGCGCTGTCTGTAACCTGACGCTGAGGCTCGAAAGTATGGTAGCAACACAGGATTAG
ATACCCCTGGTAGTCCATACCGTAAACGATGAATGCTAAAGTGTGGAGGGTTCCGCCCTCAGTGTGAGCTAACGCAT
TAAGCATCCGCGTGGGGAGTACGGGCCAAGGGCTAAACTCAAAAGGAATTGACGGGGGGCCCCACAAGCGGTGGAGC
ATGTTGTTAAATCGAAGCTACCGCAAGAACCTTACCGGCTTGTGACATACTTGCAAACTCTAAGAGATTAGACGTTCCC
TTCGGGGACATGGATAACAGGTGTGCATGGTGTCTCAGCTGTGAGATGGGGTTAAGTCCCGCAACGGCG
CAACCGTTTATTCAGTGTGCAGCATTAAGTGGGACTCTGGTAGAGACTGCGGTGACAACCGGAGGAAGGTGGGG
TGACGTCAAATCATGCCCCCTATGACCTGGCTACACAGCTGCTACAATGGATGGTACAACAGGAGTTGCGAACTCGCG
AGAGTAAGCTAATCTTAAAGCCATTCTCAGTTCGGATGTTAGGCTGCAACTCGCTCATGAAAGTCGAATCGTAGT
AATCGCGGATCAGCATGCCCGGTGAATCGTCCGGCTTGTACACCGCCCGTCACACCAGTAGAGAGTTGTAACA
CCCAAAGTC

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 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 C.H., et al 2019). The separation isolation of *L. plantarum* from Tibetan yaks was able to restrain the development of *E. coli* and *S. aureus* (Wang L., et al 2018). Few Some *Lactobacillus lactobacillus* strains appeared showed antibacterial movement against Enterobacteriaceae which that were safe to for carbapenems (CRE). This impact 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 C.C., et al 2019).

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

ACKNOWLEDGEMENTS

This paper and the The research behind this paper it 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 a scientific publications funding fund.

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). 2019 Sep; 8(9): 406.
Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakkannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer properties of *Turbinaria conoides* (J. Agardh). Clin Phytosci. 3:5

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 Davaoodabadi, Abolfazl; 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 Grela E., Kozlowska 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, Luís 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, Khayalethu 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.

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.

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

3
4
5
6
7 **Abstract.** Brown seaweeds have the potential to produce bioactive compounds. It has been shown that the bacteria-Bacteria associated
8 with 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 found bacteria were isolated, 6 of which came 6 were isolated from
11 external tissue, while 8 came from internal tissue. Through the results of an antagonistic test revealed that 7 isolates showed
12 inhibitory activity against *Staphylococcus aureus* and only 1 isolate showed the inhibition against both *S.aureus* and *E.coli*. Phenotypic
13 and genotypic identification analysis showed that the symbiont bacteria species was *Lactobacillus plantarum*.

14 **Keywords:** bioassay Bioassay, brown seaweed, 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 are obtained from seaweed, such as alginates, carrageenan,
18 and agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea GZ-et al. 2019).
19 Other 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 are particularly used in
23 the design of new antimicrobial drugs. Research for the identification of promising algal species, standardization of
24 analytical methods, isolation of compounds through integrated fractionation of bioassays, detailed chemical
25 characterization and evaluation of their safety, evaluation of synergistic effects between components, and efforts to
26 improve yields, and lowering extraction costs is needed (Marie JP et al. 2016).

27 In later decades, made strides microbiological procedures have altogether made a difference in build-up phylogenetic
28 affiliations of seaweed-related epi bacterial communities and endophytes. Be that as it may, there's inadequately
29 prove that utilitarian connections for seaweed-bacterial intuitive can be built up and well caught on. Epiphytic bacterial
30 communities are quick rapid colonizers of the ocean growth surface, some of the time versatile and able
31 to quickly metabolize algal exudates (Singh R.P and Reddy C.R.K. 2014). It has traditionally been used for children's
32 fever, as a fertilizer, repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011).
33 Seaweeds can secrete secondary metabolites with antibacterial properties (Emer S and Nisreen AG-2016). The form of
34 symbiotic mutualism occurs as Algae provide needed essential sites and nutrients, while the bacteria encourage growth
35 and protect the algal surface against symbiont bacteria isolates in as algae have abundant antimicrobial activity. The
36 existence of the bacteria is suspected as a result of infections acquired from the community (Arumugam P et al. 2017). *T.*
37 *conoides* is a tropical marine alga widely distributed in coastal waters in Asia. It here we evaluate This study evaluates the
38 properties of the brown alga *Turbinaria conoides* in producing bioactive compounds including the inhibition of human
39 pathogens (Kalaivani et al. 2016). *T. conoides* is a tropical marine alga widely distributed in coastal waters in Asia. We
40 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

41 **MATERIALS AND METHODS**

42 **Procedures**

43 **Sampling**

44 Samples of *Turbinaria* sp. (about 1 kg wet weight) were were was taken from Lima island (S: -6.001051; E:
45 106.153804) 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 meubated 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 theto 9 ml nutrient broth, to
53 produce a 10⁻¹ dilution. This was done until 10⁻⁴ dilution is produced, for each dilute nutrient broth sterile 10⁻⁴ up to 10⁻⁷.
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), (Monteiro, 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 inhibit
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 the best inhibition zone of each colony observed
119 on the plate

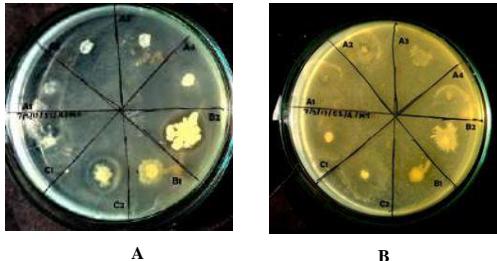
120 **** The code of number 2 identifies the isolate obtained from the second repeat

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

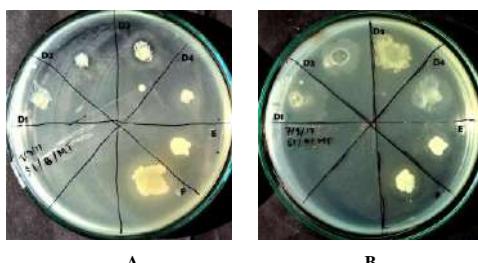
Code of isolates	Solid medium	
	Shape	Color
TUL ² -A1-2	Spread	Milky white
TUL ² -A2-2	Spread	Milky white
TUL ² -A3-2	Spread	Milky white
TUL ² -A4-2	Spread	Milky white
TUL ² -B1-2	Rhizoidal	Cloudy white
TUL ² -B2-2	Rhizoidal	Cloudy white
TUD ⁴ -C1-2	Spread	Milky white
TUD ⁴ -C2-2	Spread	Milky white
TUD 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

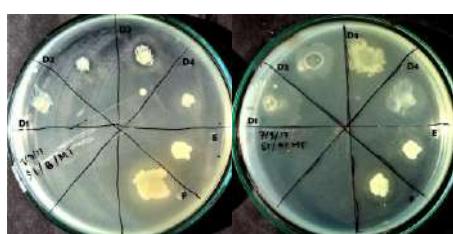


126 **Figure 1.** Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)



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 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 (Mouny B et al., 2016), while the dose of chloramphenicol

Formatted: Font: Italic

(positive control) used 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		
	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 Balouri 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 of 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 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 Kapoor et al. 2017)

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 The known characteristics of symbiont 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~~s~~, 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.), such as circular, smooth white, Gram-positive colonies
188 with brief stem cells, without shaping endospores (Davoodabadi et al. 2015).

Commented [K20]: Please rewrite this line.

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 hHighest 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.]

Commented [ND21R20]: adjusted

200 Sequens of 16S rDNA

201 GCTCAGGACGAACCGCTGGCGGCGTGCCTAAATACATGCAAGTCGAACCGAACACTCGTATTGATTGGTGCTTGCATCATGAT
202 TTACATTGAGTGAGTGAGCGAACCTGGTGGAAACCTGCCAGAAGCGGGGATAACACCTGGAAACAG
203 ATGCTAATACCGCATAACAACACTGGACGCCATGGTCGAGCTTGAAGAGATGGCTTCCGCTATCACTTTGGATGGTCCCG
204 CGCGTATTAGCTAGATGGTGGGTAACCGCTCACCTGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACA
205 TTGGGACTGAGACACCGCCCAAACCTCTAACGGGAGGCAGCAGTAGGGAATCTCCACAATGGACGAAAGTCTGATGGAG
206 CAACCGCCGTGAGTGAGAAGAGGTTTCGGCTCGTAAACACTCTGTTAAAGAAGAACATATCTGAGAGTAATGTTCA
207 GGTATTGACGGTATTAAACAGAAAGCCAAGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGTGGCAAGCGTTG
208 TCCGGATTTATTGGCGTAAAGCGAGCCAGGGCGGTTTTAAGTCTGATGTGAAAGCCTTCCGGCTCAACCGAAGAAGTG
209 CATCGGAAACTGGGAAACTTGTAGTGAGAAGAGGACAGTGGAAACTCCATGTGAGGGTGAATGCGTAGATATATGGA
210 AGAACACCCAGTGGCGAAGGGCGCTGCTGGTCTGTAACTGACGCTGAGGCTCGAAAGATGGTAGCAAACAGGATTAG
211 ATACCCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTGGAGGGTTTCGCCCTCACTGCTGAGCTAACCGAT
212 TAAGCATTCCCGCTGGGAGTACGGCCGAAGGCTGAAACTCAAAGGATTGACGGGGGCCACAAGCGGTGGAGC
213 ATGTGTTTAATTCGAAGCTACGCGAAGAACCTTACCGGCTTGTGACATACTACTGCAATCTAAGAGATTAGACGTTCCC
214 TTCGGGGACATGGATACAGGGTGCATGGTGTGCTCAGCTCGTGTGAGATGTGGGTTAAGTCCCGAACAGAGCG
215 CAACCCCTTATCATGTTGCAGCTTAAAGTGGGACTCTGGTGAAGACTGCGCTGACAACAGGAGGAAGGTGGGGA
216 TGACGTCAAATCATCATGCCCTTATGACCTGGCTACACAGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCG
217 AGAGTAAGCTAATCTCTAAAGCATTCTCAGTCCGGATTGTAGGCTGCAACTCGCTACATGAAGTCGGAATCGTAGT
218 AATCGCGGATCAGCATGCCCGTGAATCGTCCGGCTTGTACACCGCCCGTACACCATGAGAGTTGTAACA
219 CCCAAAGTC
220

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

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

231 In conclusion, *Turbinaria conoides* was is was commonly found in the gulf of Banten, Serang district, province of
232 Banten. This research showed-revealed that symbiont bacteria *Lactobacillus plantarum* are-was endophytic and potentially
233 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.

Formatted: Font: Italic

Formatted: Font: Italic

REFERENCES

- 240 Andrea GZ, Miguel A. Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara,^r 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, Kamalakkannan 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 Nisreen 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.
Microbiol*. (2020)
- 256 Kapoor Garima, Saigal Saurabh, Saigal, and Elongavan Ashok, Elongavan, 2017. Action and resistance mechanisms of
257 antibiotics: A guide for clinicians. *J Anaesthesiol Clin Pharmacol*. 2017 Jul-Sep; 33(3): 300–305.
- 258 Giovanna Romano, 2016. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Mar. Environ. res*
259 (2016)
- 260 Grela E, Kozłowska J., Grabowicka, 2018. A. Current methodology of MTT assay in bacteria-a review. *Acta Histochem.*;120(4):303–
261 311
- 262 Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. *Trends Food Sci Technol*.
263 2011;22:315–26.
- 264 Hu, C. H., Ren, L. Q., Zhou, Y., & Ye, B. C. (2019). Characterization of antimicrobial activity of three *Lactobacillus plantarum* strains isolated from
265 Chinese traditional dairy food. *Food science & nutrition*, 7(6), 1997–2005. <https://doi.org/10.1002/fsn3.1025>
- 266 Irma Esthela-Soria-Mercado, Luis Jesús Villarreal-Gómez **LJV**, Graciela Guerra-Rivas **GG**, and Nahara E. Ayala-Sánchez **NEA**, 2011.
267 Bioactive Compounds from Bacteria Associated to Marine in Algae. Biotechnology: Molecular Studies and Novel Applications for
268 Improved Quality of Human L. BoD – Books on Demand, 2012 ([25237](#))
- 269 Kalaiyani, G., Hemalatha, N., dan Poongothai. 2016. Screening Of Marinene Brown Algae Associated Potential Bacteria Producing
270 Antagonistic Bioactive Compounds Against Ut Pathogens. *International Journal of Pharma and Bio Sci*. 2016 April; 7(2): (B) 395 –
271 405. India.
- 272 Kapoor Garima-, Saigal Saurabh-, and Elongavan Ashok-, 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians.
273 *J Anaesthesiol Clin Pharmacol*. 2017 Jul-Sep; 33(3): 300–305.
- 274 Li D, Ni K, Pang H, Wang Y, Cai Y, Jin Q, 2015. Identification and Antimicrobial Activity Detection of Lactic Acid Bacteria Isolated
275 from Corn Stover Silage. *Asian-Australas J Anim Sci*. 2015 May; 28(5): 620–631.
- 276 Chen CC, Lai CC, Huang HL, Huang W-Y, Toh H-S, Weng TC, Chuang Y-C, Lu Y-C, and Tang HJ, 2019. Antimicrobial Activity
277 of *Lactobacillus* Species Against Carbapenem-Resistant Enterobacteriaceae. *Front. Microbiol*. 10:789
- 278 Manisha DM and Shyampada M, 2011. Honey: its medicinal property and antibacterial activity. *Asian Pac J Trop Biomed*. 2011 Apr;
279 1(2): 154–160.
- 280 Mari JP, Elena F, Herminia D, 2016. Antimicrobial Action of Compounds from Marine Seaweed. *Mar Drugs*. 2016 Mar; 14(3): 52.
- 281 Mezaini A, Chihib N E, Bouras A D, Arroume N N, Hornez J P, 2009. Antibacterial Activity of Some Lactic Acid Bacteria Isolated
282 from an Algerian Dairy Product. *Journal of Environmental and Public Health*. Volume 2009.
- 283 Monte, J., Abreu, A. C., Borges, A., Simões, L. C., & Simões, M. (2014). Antimicrobial Activity of Selected Phytochemicals against
284 Escherichia coli and *Staphylococcus aureus* and Their Biofilms. *Pathogens (Basel, Switzerland)*, 3(2), 473–498.
- 285 Mounyr B,*Moulay S, and Saad KI, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review *J Pharm Anal*. 2016 Apr;
286 6(2): 71–79.
- 287 Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for
288 Pharmacology. *Mar Drugs*. 2019 Aug; 17(8): 464. O'Donnell, 1993. *Fusarium and its Near Relatives*. National Center for
289 Agriculture Utilization Research, USDA, ARS, 1815 N. University Street, Peoria, Illinois, 61604, USA.
- 290 Phumudzo T, Ronald N, Khayalethu N, Fhatuwani M, 2013. Bacterial species identification getting easier. *Afr J. of Biotechnol*. Vol.
291 12(41), pp. 5975-5982
- 292 Singh R.P and Reddy C.R.K, 2014. Seaweed-microbial interactions: key functions of seaweed-associated bacteria. *FEMS Microbiol.
Ecol*, Volume 88, Issue 2, April 2014, Pages 213–230.
- 293 Smaoui S, Elleuch L, Bejar W, Karray-Rebai I, Ayadi I, Jaouadi B, Mathieu F, Chouayekh H, Bejar S, Mellouli L, 2010. Inhibition of
294 fungi and gram-negative bacteria by bacteriocin BacTN635 produced by *Lactobacillus plantarum* sp. TN635. *Appl. Biochem.
Biotechnol*. 2010 Oct; 162(4):1132-46.
- 295 Wang L, Zhang H, Rehman M.U, Khalid Mehmood K, Jiang X, Iqbal M, Tong X, Gao X, Li J, 2018. Antibacterial activity of
296 *Lactobacillus plantarum* isolated from Tibetan yaks. *J Microbial Pathogenesis*, Volume 115, Pages 293-298.
- 297 White TJ, Bruns T, Lee S, Taylor JW, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp.
298 315-322. 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, Sujulyanti, 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 inserted 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.

Tabel 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 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

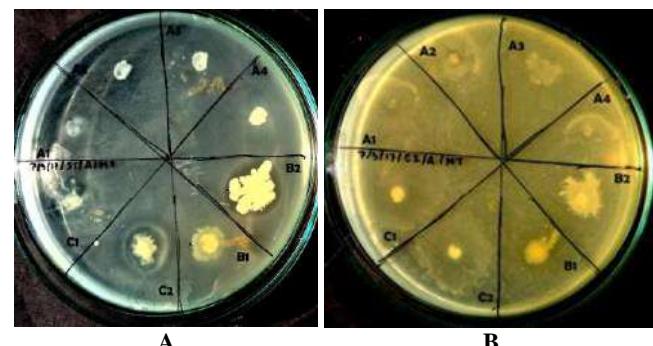


Figure 1. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *Staphylococcus aureus* (A) and *Escherichia coli* (B)

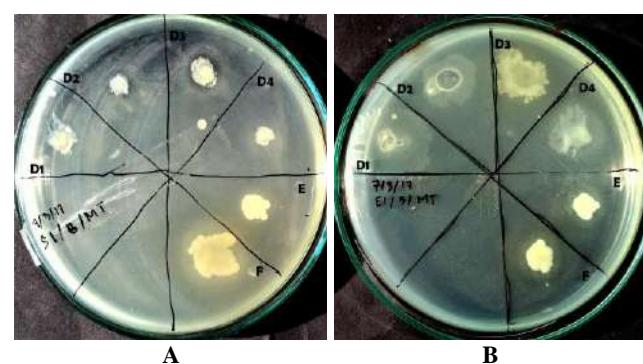


Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *Staphylococcus aureus* (A) and *Escherichia coli* (B)

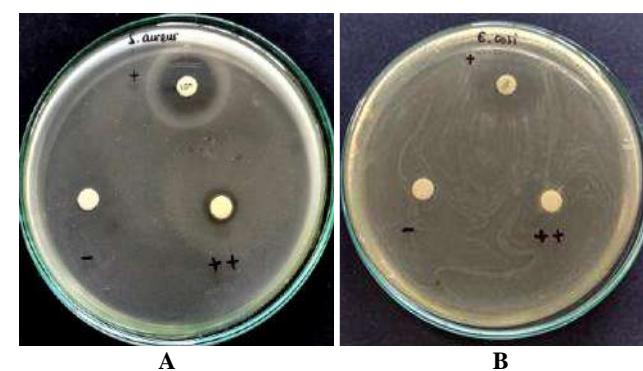


Figure 3. Results of antibiotic susceptibility test against: A. *Staphylococcus aureus* and B. *Escherichia coli*

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

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 known 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 symbiont 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 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 (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

```
GCTCAGGACGAACGGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCCTTGCATCATGATTTACATTTGAG  
TGAGTGGCGAACCTGGTGAGTAACACGTGGAAACCTGCCAGAACGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACCT  
GGACCGCATGGTCCGAGCTTGAAGAGATGGCTTCGGCTATCACTTGGATGGTCCCGCGCGTATTAGCTAGATGGTGGGGTAACGGCTCA  
CCATGGCAATGATACTGAGCCACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACCTCTACGGGAGGCAGCAGTAGG  
GAATCTCCACAATGGACGAAAGTCTGATGGAGCAACGCCCGTGAAGTGAAGAAGGGTTTCGGCTCGTAAACTCTGTGTTAAAGAAGAA  
CATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTAAACCAAGAACGCCACGGCTAACTACGTGCCAGCAGCCGGTAATACGTAGGTG  
GCAAGCGTTGTCGGATTATTGGCGTAAGCGAGCGCAGGGTTTTTAAGTCTGATGTGAAGCCTCGGCTCAACCGAAGAAGTGC  
ATCGGAAACTGGGAAACTTGAGTCAGCAGAACAGGGACAGTGGAACTCCATGTGAGCGGTAAATGCGTAGATATATGGAAGAACACCAGTGG  
CGAAGGGCGCTGTCGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGTAGCAAACAGGATTAGATACCCCTGGTAGTCCATACCGTAAA  
CGATGAATGCTAAGTGTGGAGGGTTTCCGCCCTTCAGTGCAGCTAACGCTTAAGCATTCCGCCCTGGGGAGTACGCCCGAAGGCTG  
AAACTCAAAGGAATTGACGGGGCCCGACAAGCGGGAGCATGTGGTTTAATCGAAGCTACGCGAAGAACCTTACCGGTCTTGACAT  
ACTATGCAAATCTAAGAGATTAGACGTTCCCTCGGGGACATGGGATACAGGTGTTGCACTGGTGTGAGCTCGCTGCTGAGATGTTGG  
GTTAAGTCCCACGAGCGCAACCCCTTATTATCAGTTGCCAGCATTAAGTGGCACTCTGGTAGAGACTGCCGGTACAAACCGGAGGAA  
GGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACCTCGCGAGA  
GTAAGCTAATCTCTAAAGCCATTCTCAGTTGGATTGTAAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC  
ATGCCCGGGTGAATACGTTCCCGGGCTTGTACACACCGCCCGTACACCATGAGAGTTGTAACACCCCAAAGTC
```

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, Kamalakkannan 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, Shyampada 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, Khayalethu 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* 15: 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.