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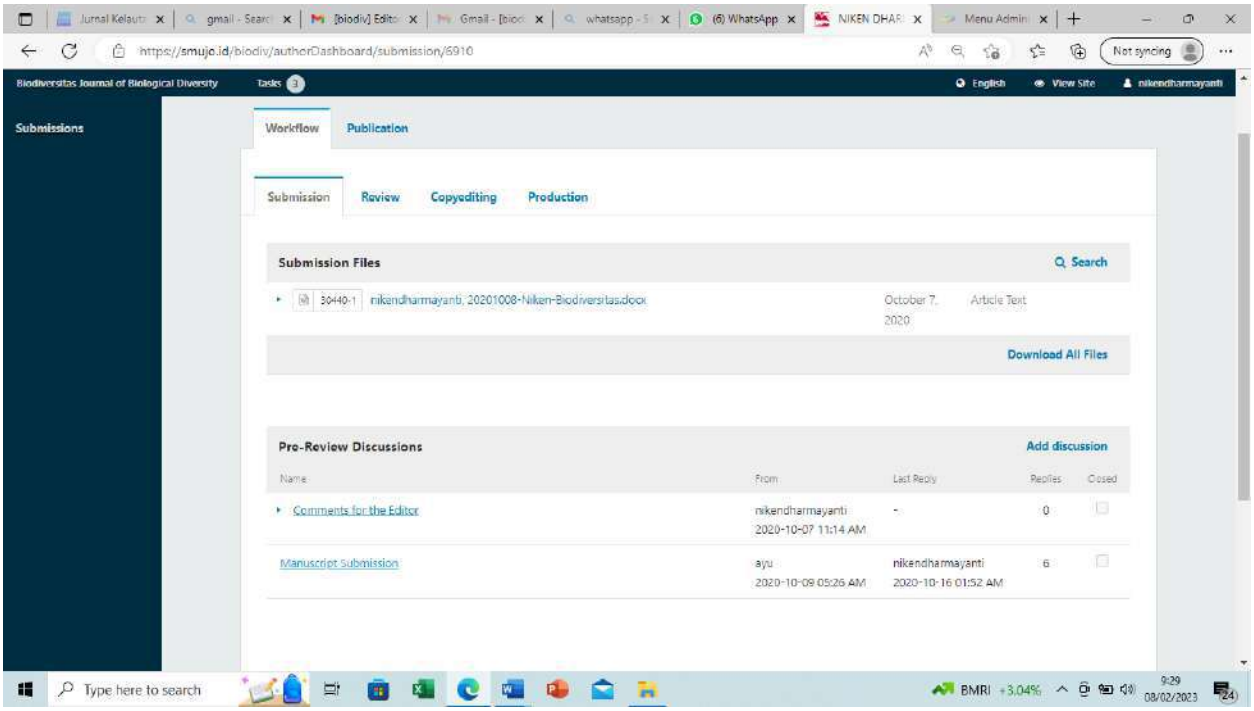

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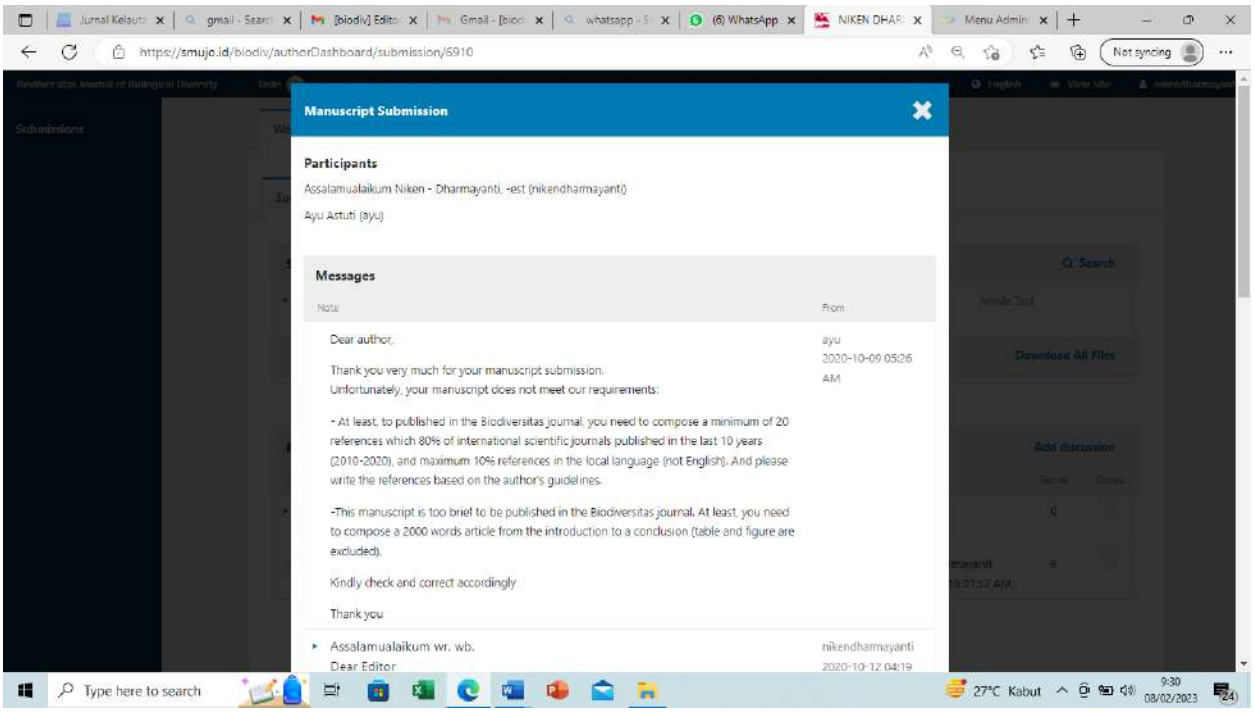

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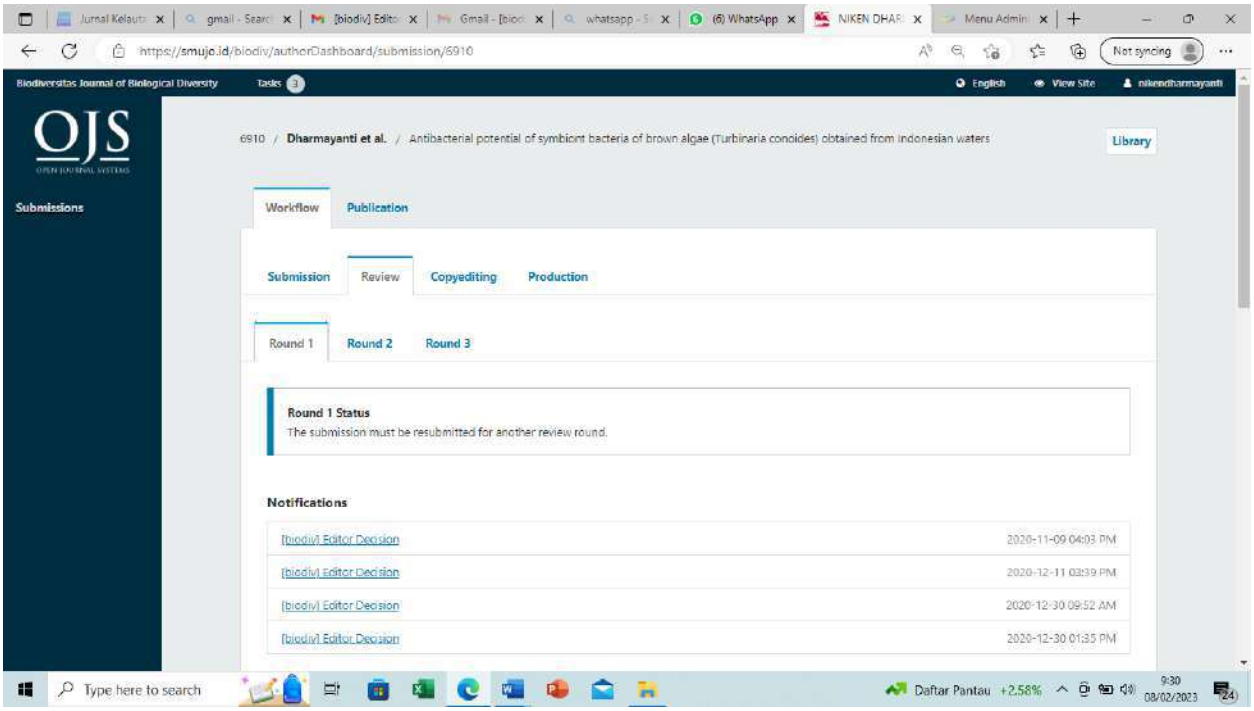
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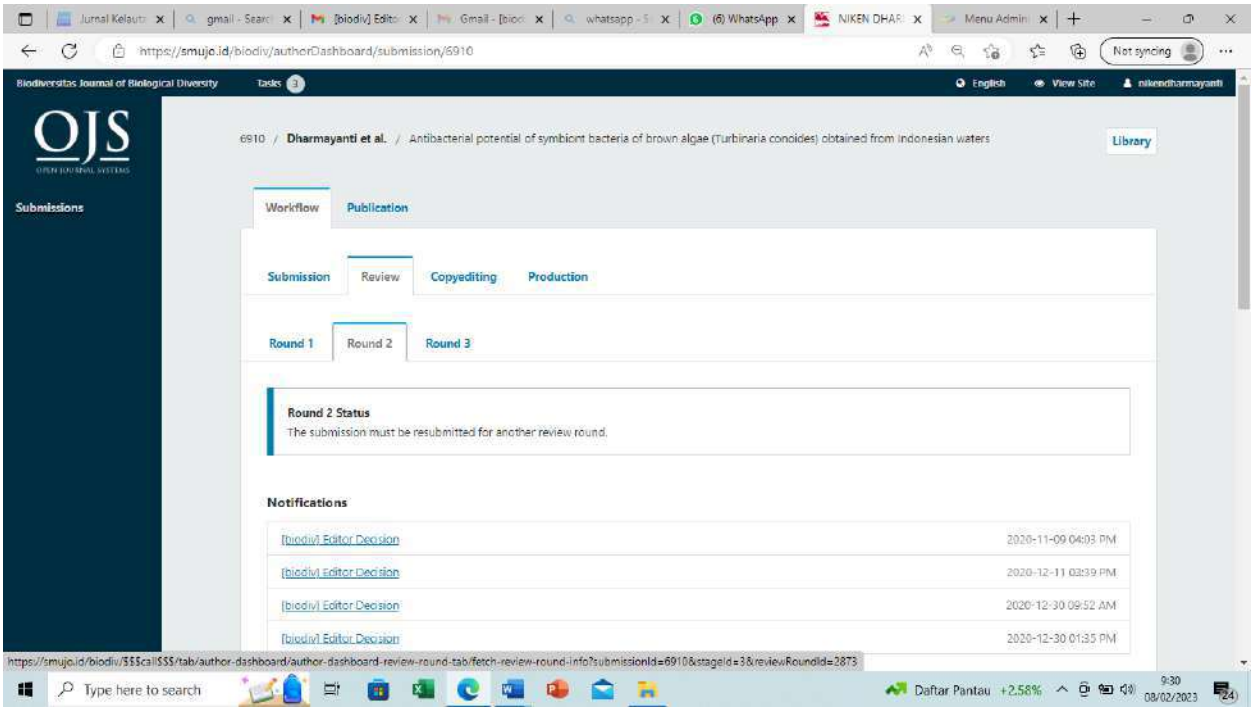
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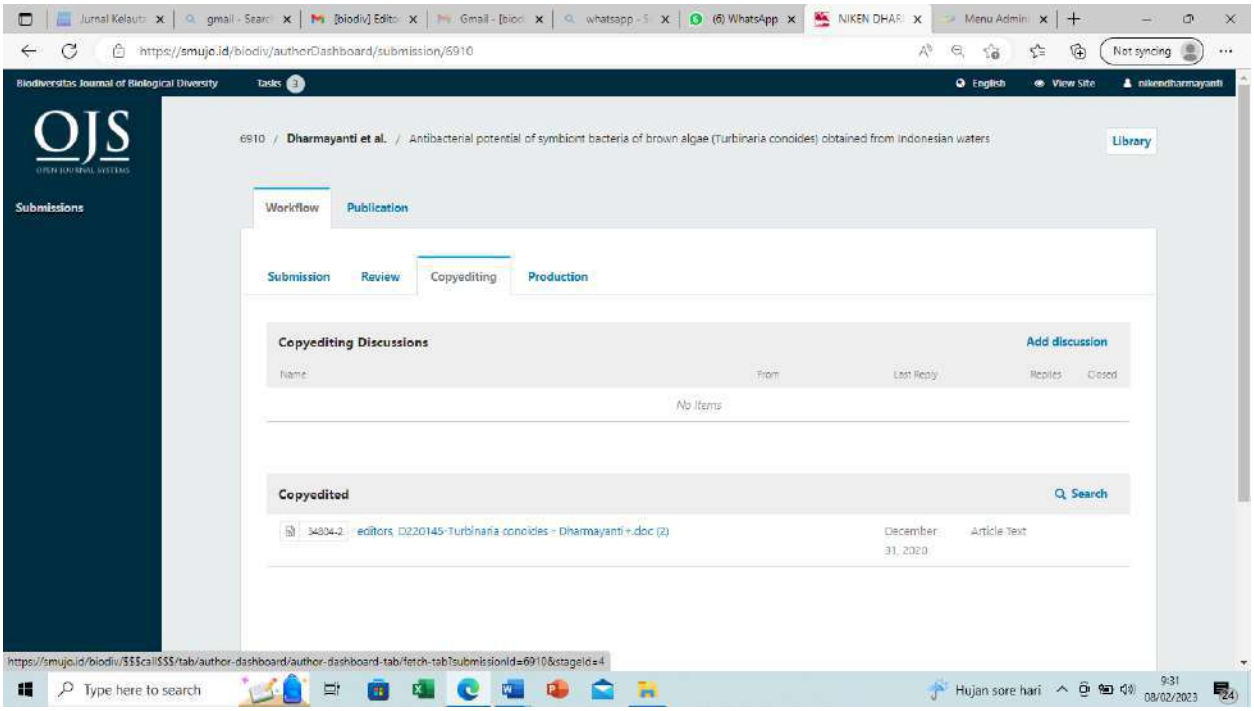

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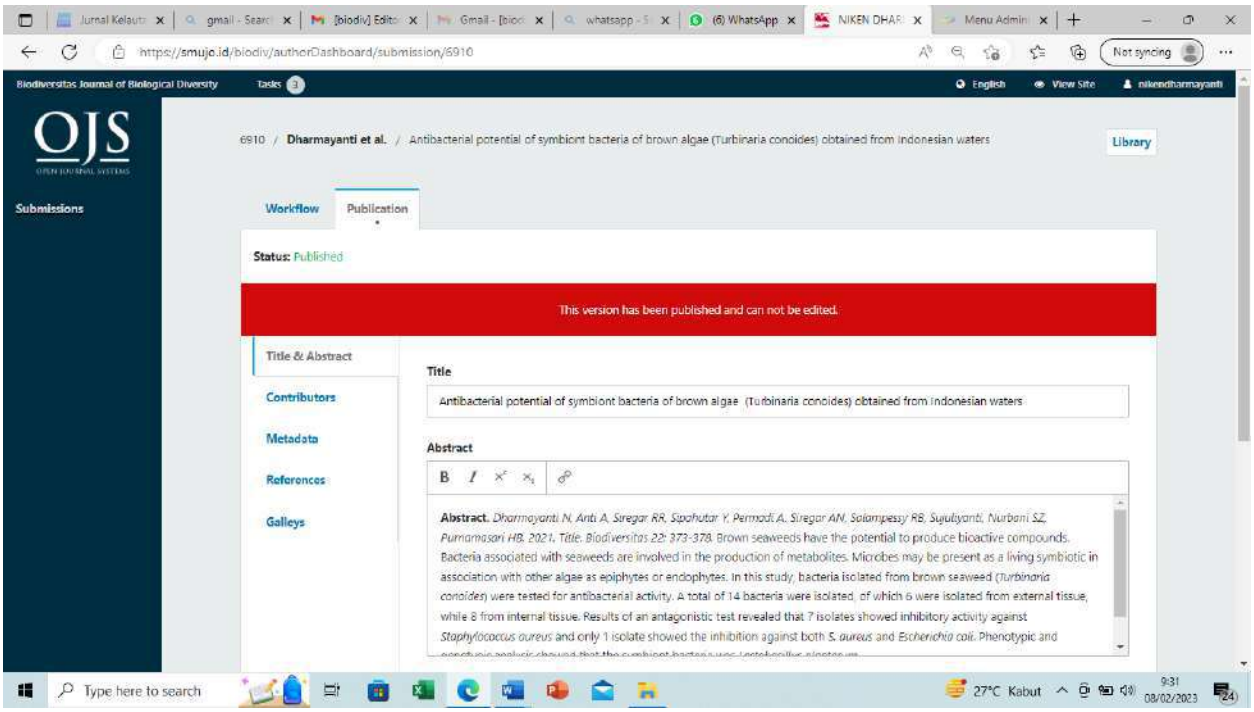

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

Title:

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

Author(s) name:

Niken Dharmayanti

Address

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

Jakarta Fisheries Tehnical University, Pasar Minggu 12520, South Jakarta, Indonesia
Phone Number: 081385058734
Email: niken.stp@gmail.com

For possibility publication on the journal:

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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.
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Jakarta, 07 October 2020

Sincerely yours,

(fill in your name, no need scanned autograph)

Niken Dharmayanti

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Antibacterials Potential Symbiont Bacteria oOf Brown Algae (*Turbinaria conoides*) Obtained fFrom ~~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, Sujuliyani, Arma ~~-~~Anti ~~anti~~

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

ABSTRACT

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

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

~~1.~~ INTRODUCTION

Seaweed ~~is an algae that lives in the sea and belongs to the division of *thallophyta*. The classification of seaweed based on pigment content consists of 4 classes, namely green seaweed (*Chlorophyta*), red seaweed (*Rhodophyta*), brown seaweed (*Phaeophyta*) and blond seaweed (*Chrysophyta*) (Suparmi and Sahri, 2009).~~ Indonesia is the largest producer of seaweed in the world (FAO 2016) cultured in nearshore coastal regions. ~~In addition to its primary economical content, the secondary metabolite content of seaweed has the~~Seaweeds ~~potential of being a producer~~produce ~~of~~ diverse bioactive metabolites with ~~vast activity as~~ antibacterial, antiviral, antifungal and cytotoxic properties (Zainuddin and Malina, 2009 in Siregar et al., 2012). ~~Bacteria usually live on a host by performing a mutually beneficial symbiosis (Sahara et al., 2013).~~ It has been shown that the bacteria associated with seaweed as epiphytes or endophytes are involved in the production of metabolites ~~that together with their host. Microbes can be present as a living symbiotic in union with various marine algae as epiphytes or endophytes~~ (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 alga*e* *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 cottoni*.

~~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~~

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

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

~~Methods~~Procedures

Sampling

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

~~**Identification and Determination of Macroalga**~~

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

Isolation of Symbiont Bacteria Producing Antibacterial Compounds

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

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

Selection of Symbiont Bacteria Isolates Antagonistically against Pathogenic Bacteria

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

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

Antibacterial Potential Testing of Symbiont Bacterial Isolate by Paper Disc Diffus~~s~~ion

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

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(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 agarr 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 awere compared up on a standardized to give a result of to assess bioactivity as sensitive, resistant, or intermediate, te then It was observed and measured its iIn each case the resistance zone where shows no colonies growth with by a ruler,was measured by using ruler to the nearest mm.

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 37ºC 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 using was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data waswere trimmed and assembled using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequencing data that has beenwere 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 morfology 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.*

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. 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 platting. 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 (Pelzcar and Chan, 1986).

Figure 1. Growth of symbiont bacteria on agar medium

The grown From 40s 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 algaealgal 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	Morphology of colonies			
		Shape	Color	Edges	Elevation
1	TUL[2]-A1-2	Round	White	Flat	Convex shiny
2	TUL[2]-A2-2	Round	White	Flat	Convex shiny
3	TUL[2]-A3-2	Round	White	Flat	Convex shiny
4	TUL[2]-A4-2	Round	White	Flat	Convex shiny
5	TUL[2]-B1-2	Round	White	Crooked	Convex shiny
6	TUL[2]-B2-2	Round	White	Crooked	Convex shiny
7	TUD[4]-C1-2	Round	White	Flat	Convex shiny
8	TUD[4]-C2-2	Round	White	Flat	Convex shiny
9	TUD[2]-D1-2	Round	White	Crooked	Convex shiny
10	TUD[2]-D2-2	Round	White	Crooked	Convex shiny
11	TUD[2]-D3-2	Round	White	Crooked	Convex shiny
12	TUD[2]-D4-2	Round	White	Crooked	Convex shiny
13	TUD[5]-E-2	Round	White	Flat	Convex shiny
14	TUD[3]-F-2	Round	White	Flat	Convex shiny

Information:
*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
** The code of isolates ([2]), ([4]), ([5]), ([3]) states isolates obtained from the dilution
*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according 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

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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 b~~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 (Dwidjoseputro, 1981).

The Selection Results Symbiont Bacteria Producing Antibacterial Compounds

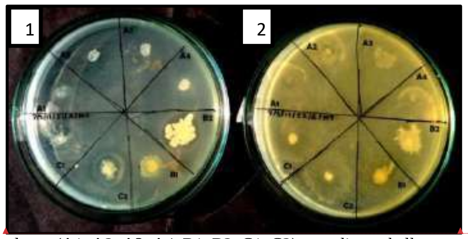

Figure 1~~2~~. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

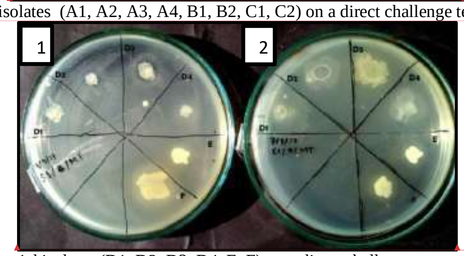

Figure 2~~3~~. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

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

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

Bacterial isolates derived from ~~the inside~~tissue showed ~~have better activity~~better inhibition than ~~bacterial~~ isolates derived from ~~the surface~~epibionts. Inhibitory zone and diameter measurement results against S.aureus and E.coli can be seen in Figure ~~3~~4 and Table ~~3~~4. 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 ≥ 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.~~

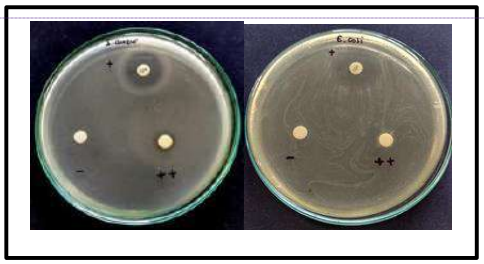

Figure 4. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

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

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

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

The area of the symptomatic supernatant inhibition zone of *S.aureus* is was 6.7 mm. According to Edrada (1998) in Kusumadewi (2004) a measured inhibition zone of less than 10 mm belongs to a veryshows weak and very active activity if and strong activity if the the inhibition zone is greater than 15 mm. Testing of antibacterial activity of the symbiont bacteria supernatant obtained was still far from the results of the antibiotic activity of the tested comparatochloramphenicol r control. This is because the antibacterial compound of the applied extracted symbiont bacteria is stillwas a supernatant with thecontaining secondary metabolites. it contains, butHowever, the test results have indicated the presence of provide clear evidence of antibacterial activity. Generally the chemical structure of metabolites from marine products is often different from the secondary differs from those of terrestrial origin metabolite of land (Gudbjarnason 1999 in Nofiani, 2005). Seawater contains an active inhibitor agent for Gram positive bacteria, according to Okami (1982)2) in in Nofiani (2005) that seawater contains an active inhibitor agent for organisms, seawater has the ability of inhibitors a gainst 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 (Burgesset 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 effectsors, 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 haswere rod shaped, non acidic, non spore forming, non motile, aerobically grown, negative catalase, and positive to carbohydrate

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tests. In general, the identification of microscopically selected isolates showeds specific characteristics possessed byof lactic acid bacteria (*Lactobacillus* spp.), such as round colonies, milky white, Gram positive with short stem cells, and doeswithout not formforming endospores (Desniar 2012 in Saskia, 2014). The genus Lactobacillus can be isolated from several different habitats, eg from milkfish intestine (Sulistijowati and Mile, 2015), bekasam products (Ingratubun 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 ≥ 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%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACAT
TTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCG
CATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATG
GTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAA
CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTC
GGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTA
ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
AAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC
CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTC
GAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCT
TCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTA
GACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG
ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAG
CTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC
ATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTC

Figure 4. Sequens of 16S rDNA

Genotypic identification of symbiont bacteria were using DNA encoding the 16S rDNA gene to determine genus and strain. 16S rDNA can be used as a molecular marker for species definition because this molecule exists in every organism with a function identical to all organisms. Data offor base sequence encoding gene of 16S rDNA can be seen in figure 4, it 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*,

4. CONCLUSION

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

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

REFERENCES

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 and Anamaya Publishers, New Delhi, India.

Cowan, S.T and Steel, K.J. 1993. *Manual for the Identification of Medical Bacteria*. 3rd Edition. University of Cambridge. UK.

Dwidjoseputro, D. 1981. *Dasar-dasar Mikrobiologi*. Cetakan ke-5. Djambatan, 1981: Jakarta.

Hudzicki, 2009. *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, ASM Microbelibrary*. American Society for Microbiology, http://www.microbelibary.org/component/resource/laboratory-test/3189-kirby-bauerdisk-diffusion-susceptibility-test-protocol., (7/04/2014)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Dear **Editor-in-Chief,**

I herewith enclosed a research article,

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

Author(s) name:

Niken Dharmayanti

Address

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Phone Number: 081385058734
Email: niken.stp@gmail.com

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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.

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Niken Dharmayanti

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Antibacterials Potential Symbiont Bacteria oOf Brown Algae (*Turbinaria conoides*) Obtained fFrom ~~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, Sujuliyani,~~ Arpan Nasri Siregar, Randi Bokhi Salampessy, Sujuliyani, Siti Zachro Nurbani, Heni Budi PurnamasariArma Anti anti

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

ABSTRACT

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

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

~~1.~~ INTRODUCTION

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

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

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

~~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 alga~~e~~ *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 cottoni*.

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

~~2.~~ MATERIALS AND METHODS

~~Materials~~

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

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

~~Methods~~Procedures

Sampling

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

~~Identification and Determination of Macroalga~~

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

Isolation of Symbiont Bacteria Producing Antibacterial Compounds

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

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

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

Selection of Symbiont Bacteria Isolates Antagonistically against Pathogenic Bacteria

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

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

Antibacterial Potential Testing of Symbiont Bacterial Isolate by Paper Disc Diffussion

Testing inhibitory the supernatant of symbiont bacteria on thefor inhibitory growth of *E.coli* and *S.aureus* was performed by the agar diffusion method ((Hudzicki, 2009Grela E et al. 2018) REFERENCE). Supernatant was obtained by separating the filtrate and supernatant by centrifugation processcentrifuge for 1 hour , temperature at (25 ºC and 3000 rpm). Paper discs containing supernatant 40 µL and the negative control nutrient broth 40 µL which has allowed were dried left for 1 hour to reduce the water excess (dried), negative control nutrient broth 40 µL also has allowed for 1 hour to reduce the water excess (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 agarr 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 awere compared up on a standardized to give a result of to assess bioactivity as sensitive, resistant, or intermediate, te then It was observed and measured its iIn each case the resistance zone where shows no colonies growth with by a ruler.was measured by using ruler to the nearest mm.

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 (Phumudzo T, 2013Lay, 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 37ºC 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 using was performed with an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data waswere trimmed and assembled using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequencing data that has beenwere assembled in BLAST with genomic data that has been registered in DDBJ / DNA Data Bank of Japan (http://blast.ddbj.nig.ac.jp/)

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~~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 morfology 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 platting. Observation of medium incubated with temperature 37ºC 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 (Pelzcar 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 algae~~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	Morphology of colonies			
		Shape	Color	Edges	Elevation

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1	TUL^2-A1-2	Round	White	Flat	Convex shiny
2	TUL^2-A2-2	Round	White	Flat	Convex shiny
3	TUL^2-A3-2	Round	White	Flat	Convex shiny
4	TUL^2-A4-2	Round	White	Flat	Convex shiny
5	TUL^2-B1-2	Round	White	Crooked	Convex shiny
6	TUL^2-B2-2	Round	White	Crooked	Convex shiny
7	TUD^4-C1-2	Round	White	Flat	Convex shiny
8	TUD^4-C2-2	Round	White	Flat	Convex shiny
9	TUD^2-D1-2	Round	White	Crooked	Convex shiny
10	TUD^2-D2-2	Round	White	Crooked	Convex shiny
11	TUD^2-D3-2	Round	White	Crooked	Convex shiny
12	TUD^2-D4-2	Round	White	Crooked	Convex shiny
13	TUD^5-E-2	Round	White	Flat	Convex shiny
14	TUD^3-F-2	Round	White	Flat	Convex shiny

Information:
*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
** The code of isolates (^{2}), (^{4}), (^{5}), (^{3}) states isolates obtained from the dilution
*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according 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 ~~3~~2. Identification of the isolates on slant agar

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

~~Observations of bacteria can be done individually or in groups in the form of colonies. If the b~~Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is different for each species and it is characteristic of a particular species (Erin RS~~Dwidjoseputro, 1981~~2012).

The Selection Results Symbiont Bacteria Producing Antibacterial Compounds

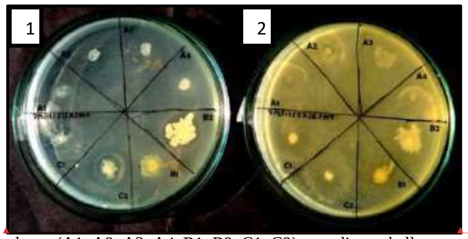

Figure 1~~2~~. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

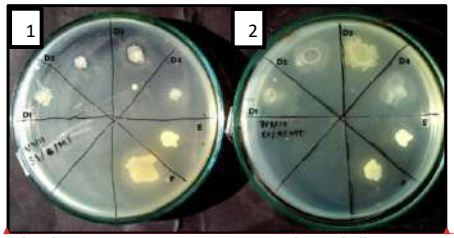

Figure 23. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

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* ~~is~~ was not as good as its inhibition against *S.aureus*.

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

Bacterial isolates derived from ~~the inside~~tissue showed ~~have better activity~~better inhibition than ~~bacterial –~~isolates derived from ~~the surface~~epibionts. Inhibitory zone and diameter –measurement results against S.aureus and E.coli can be seen in Figure ~~3~~4 and Table ~~3~~4. 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.~~Lay, 1994~~2016), while the dose of chloramphenicol (positive control) used is lower at 0.01 mg, so it can be said that bacteria Test is sensitive to positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that a supernatant still containing medium has no effect on the activity formed. From the stability of the measured inhibition zone, the antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram positive bacteria and are merely bacteriostatic for Gram negative bacteria. Paper disc with a supernatant applied to a Gram positive bacterial plate indicates a stable clear zone even after a 48-hour incubation period. While against Gram negative bacteria, around the disc paper shows the presence of inhibitory activity but gradually become turbid before the incubation period reaches 24 hours.

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

~~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~~ (20141) the inner symbiotic bacteria generally have abundant populations and are specific microbes

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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 contr~~ ~~es of test bacteria with 16.8 mm inhibition against *S.* col with a concentration of 0.03 mg on a paper disc i~~ ~~mm (Lay, 1994), while the dose of chloramphenicol~~ ~~can be said that bacteria Test is sensitive to positive~~ ~~bacterial inoculation) indicates the absence of acti~~ ~~at a supernatant still containing medium has no effe~~

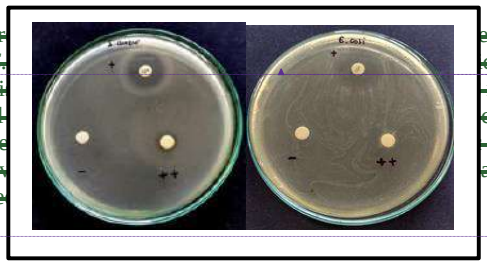

Figure 43. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

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

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

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

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

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~~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~~ in Nofiani (~~2005~~2017) ~~that seawater contains an active inhibitor agent for organisms, seawater has the ability of inhibitors a gainst 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 (Burgesset 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 effectsors, 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 ~~has~~were 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 showeds specific characteristics ~~possessed by~~of lactic acid bacteria (*Lactobacillus* spp.), such as round colonies, milky white, Gram positive with short stem cells, ~~and does~~without ~~not form~~forming endospores (~~Desniar 2012 in Saskia,~~ Davoodabadi et al. ~~2014~~2015). ~~The genus Lactobacillus can be isolated from several different habitats, eg from milkfish intestine (Sulistijowati and Mile, 2015), bekasam products (Ingratubun 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 ≥ 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%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACAT
TTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCG
CATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATG
GTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAA
CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTC
GGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTA
ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
AAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC
CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTC
GAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCT
TCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTA
GACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG
ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAG
CTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC
ATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTC

Figure 4. Sequens of 16S rDNA

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Genotypic identification of symbiont bacteria were using DNA encoding the 16S rDNA gene to determine genus and strain. 16S rDNA can be used as a molecular marker for species definition because this molecule exists in every organism with a function identical to all organisms. Data offor base sequence encoding gene of 16S rDNA can be seen in figure 4, it 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*.

4. CONCLUSION

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

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

ACKNOWLEDGEMENTS

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

REFERENCES

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

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, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant and anticancer properties of *Turbinaria conoides* (J. Agardh). Clin Phytosci. (2017) 3:5

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

Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015. Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Pubmed.

Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological mechanisms and Applications. Mar Drugs. (2016) Apr; 14(4): 81.

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

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

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.

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

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

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

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

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 Sci. 2016 April; 7(2): (B) 395 – 405. India.

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

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

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 sources: revisiting our understanding. J. of Appl. Phycol. volume 29 pages 949–982 (2017)

Mounyr 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.

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

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

White TJ, Bruns T, Lee S, 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 Aplications, Academic Press, Inc., New York.

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 and Anamaya Publishers, New Delhi, India.

Cowan , S.T and Steel , K.J. 1993. *Manual for the Identification of Medical Bacteria*. 3rd Edition. University of Cambridge. UK.

Dwidjoseputro, D. 1981. *Dasar-dasar Mikrobiologi*. Cetakan ke-5. Djambatan, 1981: Jakarta.

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Hudzicki. 2009. *Kirby Bauer Disk Diffusion Susceptibility Test Protocol, ASM Microbelibrary*. American Society for Microbiology. http://www.microbelibary.org/component/resource/laboratory-test/3189-kirby-bauerdisk-diffusion-susceptibility-test-protocol., (7/04/2014)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

INTRODUCTION

Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse, and unique compounds (Bahare S et al. 2019). Many are the substances obtained from seaweed, such as alginates, carrageenan, and agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea GZ et al. 2019). Other compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory, and antifouling activity. Therefore, seaweed can provide promising bioactive that can be used in the treatment of human diseases or new antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. Much attention has been paid to developing innovative projects for pharmaceuticals. Seaweed applications, especially 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).

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

MATERIALS AND METHODS

Procedures

Sampling

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

Isolation of symbiont bacteria producing antibacterial compounds

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

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

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

Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria

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

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

Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion

Testing the supernatant of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was performed by the agar diffusion method (Grela E et al. 2018)). The supernatant was obtained by separating the filtrate and supernatant by centrifuge for 1 hour (25 ºC and 3000 rpm). Paper discs containing supernatant 40 µL and the negative control nutrient broth 40 µL were left for 1 hour to reduce the water excess, and positive control chloramphenicol 0.01 mg/mL, 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. If the organism is killed or inhibited by both the supernatant and chloramphenicol as an 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 to assess bioactivity as sensitive, resistant, or intermediate, in each case the resistance zone where shows no colonies growth was measured by using a ruler to the nearest mm.

Identification of phenotype and genotype of symbiont bacteria

General bacterial identification (Phumudzo T, 2013) followed colony characteristic observations on liquid medium and solid medium, observing cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and 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 37ºC 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 *Turbinaria conoides.* Determination of the type of bacteria was performed based on ???. Symbiont bacteria species were determined by molecular testing.

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The DNA of the symbiont bacteria isolateds 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. After 30 cycles completed, Ffollowed 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

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 epibionts, while the other 8 came from the algal tissue. The results of identification of colonies grown on mixed cultures can be seen in Table 1. and identification of isolates isolated into slant agar can be seen in Table 2.

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

Information:
*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
** The code of isolates ([2]), ([4]), ([5]), ([3]) states isolates obtained from the dilution
*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according 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. 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

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

The Selection Results Symbiont Bacteria Producing Antibacterial Compounds

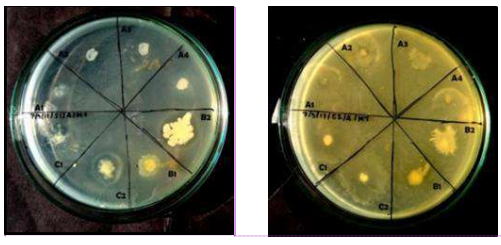

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Figure 1. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

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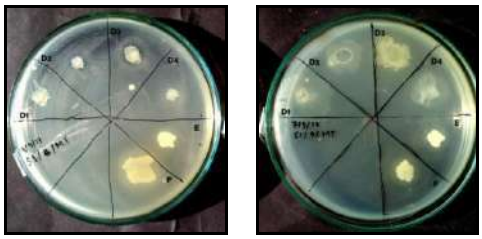

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Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

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.

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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.

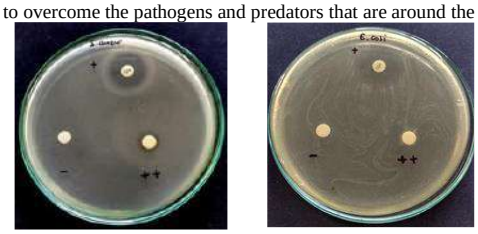

Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

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).

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

The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Mounyr Balouiri 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 testsing, 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%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTA
CATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATA
CCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAG
ATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCC
AAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTT
TCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGC
TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTT
TTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAAC
TCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGC
TCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCC
CTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATT
AGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAA
GCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAG
CATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTC

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

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

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, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant and anticancer properties of *Turbinaria conoides* (J. Agardh). Clin Phytosci. (2017) 3:5

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

Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015. Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Pubmed.

Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological mechanisms and Applications. Mar Drugs. (2016) Apr; 14(4): 81.

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

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

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.

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

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

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

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

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 Sci. 2016 April; 7(2): (B) 395 – 405. India.

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

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

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 sources: revisiting our understanding. J. of Appl. Phycol. volume 29 pages 949–982 (2017)

Mounyr 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.

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

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

White TJ, Bruns T, Lee S, 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 Aplications, Academic Press, Inc., New York.

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

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

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

INTRODUCTION

Seaweed is one of the largest producers of biomass from the ocean and is a potential source of new, diverse, and unique compounds (Bahare S et al. 2019). Many are the substances obtained from seaweed, such as alginates, carrageenan, and agar, which have been used for decades in traditional medicine, pharmacology, and food (Andrea GZ et al. 2019). Other compounds have bacteriostatic or antibacterial, antiviral, anti-tumor, anti-inflammatory, and antifouling activity. Therefore, seaweed can provide promising bioactive that can be used in the treatment of human diseases or new antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry. Much attention has been paid to developing innovative projects for pharmaceuticals. Seaweed applications, especially 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).

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

MATERIALS AND METHODS

Procedures

Sampling

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

Isolation of symbiont bacteria producing antibacterial compounds

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

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

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

Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria

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

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

Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion

Testing the supernatant of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was performed by the agar diffusion method (Grela E et al. 2018)). The supernatant was obtained by separating the filtrate and supernatant by centrifuge for 1 hour (25 ºC and 3000 rpm). Paper discs containing supernatant 40 µL and the negative control nutrient broth 40 µL were left for 1 hour to reduce the water excess, and positive control chloramphenicol 0.01 mg/mL, 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. If the organism is killed or inhibited by both the supernatant and chloramphenicol as an 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 to assess bioactivity as sensitive, resistant, or intermediate, in each case the resistance zone where shows no colonies growth was measured by using a ruler to the nearest mm.

Identification of phenotype and genotype of symbiont bacteria

General bacterial identification (Phumudzo T, 2013) followed colony characteristic observations on liquid medium and solid medium, observing cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and 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 37ºC 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 *Turbinaria conoides.* Determination of the type of bacteria was performed based on ???. Symbiont bacteria species were determined by molecular testing.

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The DNA of the symbiont bacteria isolateds 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. After 30 cycles completed, Ffollowed 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

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 epibionts, while the other 8 came from the algal tissue. The results of identification of colonies grown on mixed cultures can be seen in Table 1. and identification of isolates isolated into slant agar can be seen in Table 2.

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
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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

Information:
*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
** The code of isolates ([2]), ([4]), ([5]), ([3]) states isolates obtained from the dilution
*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according 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. 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
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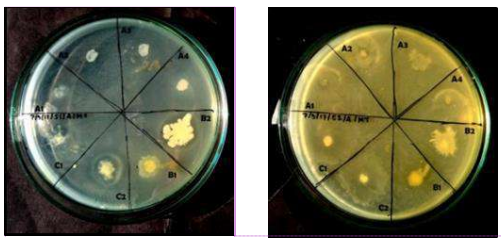

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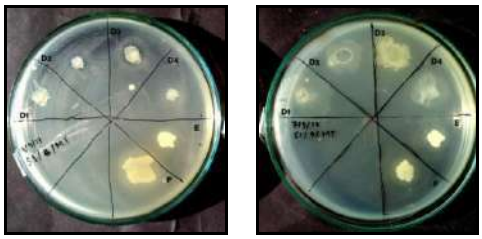

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The antibacterial compounds produced by symbiont bacteria isolates showed different inhibitory activity against both tested bacteria *S.aureus* and *E.coli*. According to Irma ESM (2011) the inner symbiotic bacteria generally have abundant populations 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.

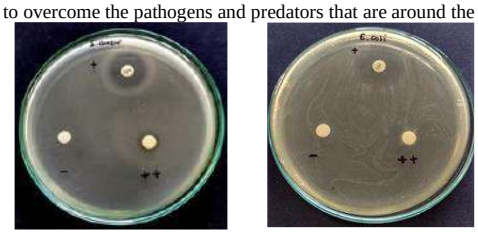

Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

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).

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

The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Mounyr Balouiri 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 testsing, 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%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTA
CATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATA
CCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAG
ATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCC
AAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTT
TCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGC
TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTT
TTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAAC
TCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGC
TCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCC
CTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATT
AGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC
GAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAA
GCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAG
CATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTC

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

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

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, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant and anticancer properties of *Turbinaria conoides* (J. Agardh). Clin Phytosci. (2017) 3:5

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

Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015. Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Pubmed.

Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological mechanisms and Applications. Mar Drugs. (2016) Apr; 14(4): 81.

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

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

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.

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

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

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

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

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 Sci. 2016 April; 7(2): (B) 395 – 405. India.

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

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

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 sources: revisiting our understanding. J. of Appl. Phycol. volume 29 pages 949–982 (2017)

Mounyr 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.

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

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

White TJ, Bruns T, Lee S, 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 Aplications, Academic Press, Inc., New York.

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

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

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

INTRODUCTION

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

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

MATERIALS AND METHODS

Procedures

Sampling

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

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Isolation of symbiont bacteria producing antibacterial compounds

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 incubated with 30 mL nutrient broth ~~nutrient~~ medium and shaken at room temperature for 24 hours.

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

Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria

~~To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria~~ For this, a qualitative test was ~~conducted~~ 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 ~~J~~, et al 2014)). The media ~~were~~ was then incubated for 48 hours at 37 °C. Each scratching round of isolates was then marked by a unique code.

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

Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion

Antibacterial ~~Testing~~ testing ~~the supernatant~~ of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was performed by the agar diffusion method (Grela ~~E~~ et al. 2018) ~~)~~. The supernatant was obtained by separating the filtrate and supernatant ~~by~~ was centrifuge for 1 hour (25 ~~°~~°C and 3000 rpm). Paper discs containing 40 µL supernatant was considered as the treatment ~~40 µL and~~ while 40 µL nutrient broth was used in ~~the~~ negative control ~~nutrient broth 40 µL were left for 1 hour to reduce the water excess,~~ and chloramphenicol (0.01 mg/mL) was used as positive control. ~~chloramphenicol 0.01 mg/mL,~~ 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. ~~If the organism is killed or inhibited by both the supernatant and chloramphenicol as an antibiotic positive control, there will be no growth in the immediate area around the disc, this is called the zone of inhibition~~. 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. ~~in each case the resistance zone where shows no colonies growth was measured by using a ruler to the nearest mm.~~

Identification of phenotype and genotype of symbiont bacteria

General bacterial identification was carried out on the basis of colony characteristic observations on liquid medium and solid medium ~~(Phumudzo T, 2013) followed colony characteristic observations on liquid medium and solid medium, followed by observing~~ cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and ~~Biochemistry~~ biochemical test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase) as described by Phumudzo, (2013). The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *Turbinaria conoides* samples on the agar medium ~~in pour plating~~. ~~Observation of medium~~The plates were incubated ~~with~~ 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 from the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from *Turbinaria conoides*. ~~Determination of the type of bacteria was performed based on Phenotype and Genotype~~ ~~Symbiont bacteria species were determined by molecular testing.~~

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~~ 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/)

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RESULTS AND DISCUSSION

The Result of Symbiont Bacteria Isolation

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

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

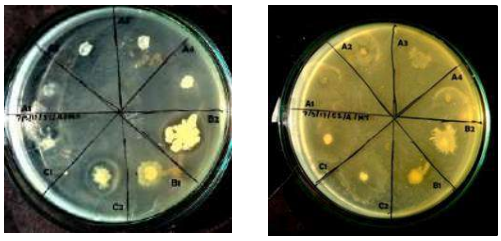
Note:
*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
** The code of isolates ([2]), ([4]), ([5]), ([3]) states isolates obtained from the dilution
*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient 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. ~~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

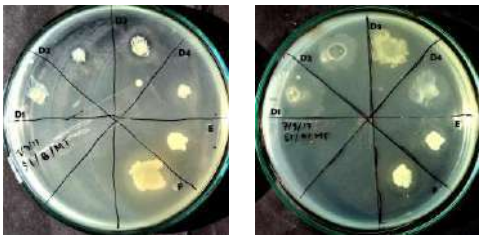
~~Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is different for each species and it is characteristic of a particular species (Erin RS 2012).~~ Bacteria were isolated in a solid medium and the size of the colony was different for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

The selection results symbiont bacteria producing antibacterial compounds



A **B**

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)



A **B**

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

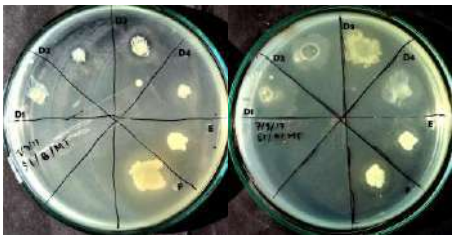


Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

~~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*.~~ 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*.

~~Isolates with showing inhibition were re-selected by looking at the best and largest clear zone.~~ 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~~ 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*~~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 ~~B~~ et al., 2016), while the dose of chloramphenicol

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(positive control) used ~~is~~ was ~~lower at~~ less than 0.01 mg, so it can be said that bacteria ~~Test~~ test ~~is~~ was found to be sensitive to the positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that ~~a~~ supernatant ~~still containing medium~~ has no effect on the activity formed. ~~From the stability of the measured inhibition zone, the~~ The antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram-positive bacteria and ~~are~~ were merely bacteriostatic for Gram-negative bacteria. Paper disc with ~~a~~ supernatant applied to a Gram-positive bacterial plate indicate~~s~~ 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.

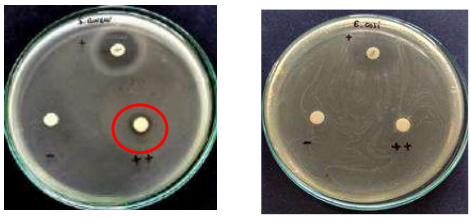

Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

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

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

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

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

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

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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.

Sequens of 16S rDNA

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGAT
TTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAG
ATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCG
CGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACA
TTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAG
CAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCA
GGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTG
TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTG
CATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGA
AGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAG
ATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCAT
TAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCC
TTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGA
TGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCG
AGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGT
AATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACA
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 publication~~s~~ 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, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer properties of *Turbinaria conoides* (J. Agardh). Clin Phytosci. 3:5

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

Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015. Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Pubmed.

Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological mechanisms and Applications. Mar Drugs. (2016) Apr; 14(4): 81.

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

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

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

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

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

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

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

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

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 Sci. 2016 April; 7(2): (B) 395 – 405. India.

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 of *Lactobacillus* Species Against Carbapenem-Resistant *Enterobacteriaceae*. *Front. Microbiol.* 10:789

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

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

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

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

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

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

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.

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, Volume 115, Pages 293-298. ,

White TJ, Bruns T, Lee S, 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.

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

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

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

INTRODUCTION

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

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

MATERIALS AND METHODS

Procedures

Sampling

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

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Isolation of symbiont bacteria producing antibacterial compounds

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 ~~incubated with~~ put into a 30 ml~~I~~ nutrient broth ~~nutrient~~ medium and shaken at room temperature for 24 hours.

After the extraction process, 1 ml of the refreshed samples from ~~in~~ the 30 ml broth nutrient was measured out and homogenized in the sterile test tube containing 9 ml of ~~medium were diluted in stages in~~ sterile ~~the~~to 9 ml nutrient broth. to produce a 10^{-1} dilution. This was done until 10^{-5} dilution is produced. ~~for each dilute nutrient broth sterile 10^{-1} up to 10^{-5}.~~ Each dilution was grown on a plate count agar medium by incubating them at 37 °°C for 2 x 24 hours.. ~~After incubating the petri dishes which contained samples from each dilution, then the colonies bacteria from alga would appear~~. ~~The colonies~~ Colonies of bacteria ~~producing~~ that produce antimicrobial compounds were characterized by a clear zone ~~around the colonies~~. Furthermore, the colonies with stable inhibition zones were collected by isolating them on a slant agar medium., ~~with a clear code~~.

Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria

~~To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria~~ For this, a qualitative test was ~~conducted~~ 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 ~~J~~, et al 2014)). The media ~~were~~ was then incubated for 48 hours at 37 °C. Each scratching round of isolates was then marked by a unique code.

Inhibition zones were ~~determined as those~~ showing clear zones around the colony of symbiont bacteria isolates~~,~~ for both *Escherichia coli* and *Staphylococcus aureus*. Strains that showed maximum antagonistic effect against tested pathogens were identified. ~~These were isolated and selected for further antibacterial testing by paper disc and identification of phenotype and genotype~~. 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~~ testing ~~the supernatant~~ of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was performed by the ~~agar~~ paper disc diffusion method ~~(~~Grela ~~E~~ et al. 2018)~~)~~. The supernatant was obtained by separating the filtrate and the supernatant ~~by~~ was centrifuged for 1 hour (25 °°C and 3000 rpm). Paper discs containing 40 µL supernatant was considered as the treatment ~~40 µL and~~ while 40 µL nutrient broth was used in ~~the~~ negative control ~~nutrient broth 40 µL were left for 1 hour to reduce the water excess,~~ and chloramphenicol (0.01 mg/mL) was used as a positive control. ~~chloramphenicol 0.01 mg/mL,~~ 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. ~~If the organism is killed or inhibited by both the supernatant and chloramphenicol as an antibiotic positive control, there will be no growth in the immediate area around the disc, this is called the zone of inhibition~~. 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. ~~in each case the resistance zone where shows no colonies growth was measured by using a ruler to the nearest mm.~~

Identification of phenotype and genotype of symbiont bacteria

General bacterial identification was carried out based on ~~on the basis of~~ colony characteristic observations on liquid medium and solid medium ~~(Phumudzo T, 2013) followed colony characteristic observations on liquid medium and solid medium,~~ followed by ~~observing~~ cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and ~~Biochemistry~~ biochemical test (motility, gelatin hydrolysis, citrate, urease, carbohydrates, and catalase) as described by Phumudzo, (2013). The initial selection of isolates from mixed cultures was carried out after enrichment and planting of *Turbinaria conoides* samples on the agar medium ~~in pour plating~~. ~~Observation of medium~~The plates were incubated ~~with~~ 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 from the bacterial isolate characterization were used to estimate the type of symbiotic bacteria isolated from *Turbinaria conoides*. ~~Determination of the type of bacteria was performed based on Phenotype and Genotype Symbiont bacteria species were determined by molecular testing~~.

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~~ 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

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(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. ~~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 epibionts, while the other 8 came from the algal tissue. The results of the identification of colonies grown on mixed cultures can be seen in Table 1, and identification of isolates isolated into slant agar can be seen in Table 2.~~ The macroscopic results of colonies on mixed cultures can be seen in Table 1, and on slant agar can be seen in Table 2.

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

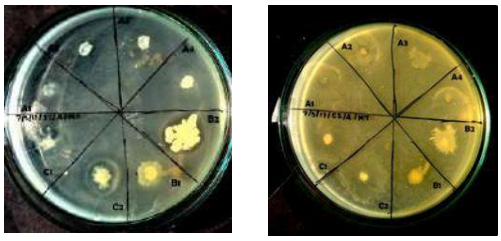
Note:
*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
** The code of isolates ([2]), ([4]), ([5]), ([3]) states isolates obtained from the dilution
*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient 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. ~~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

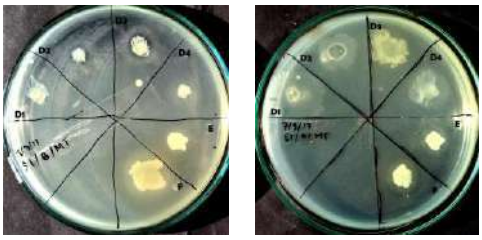
~~Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is different for each species and it is characteristic of a particular species (Erin RS 2012).~~ Bacteria were isolated in a solid medium and the size of the colony was different for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

The selection results symbiont bacteria producing antibacterial compounds



A **B**

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)



A **B**

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

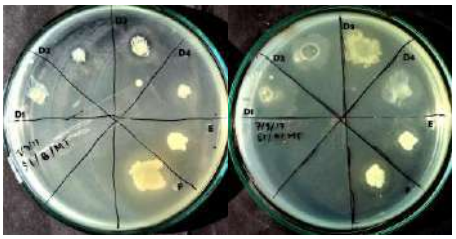


Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

~~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*.~~ 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*.

~~Isolates with showing inhibition were re-selected by looking at the best and largest clear zone.~~ 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~~ 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*~~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 ~~B~~ et al., 2016), while the dose of chloramphenicol

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(positive control) used ~~is~~ was ~~lower at~~ less than 0.01 mg, so it can be said that bacteria ~~Test~~ test ~~is~~ was found to be sensitive to the positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that ~~a~~ supernatant ~~still containing medium has no effect on~~does not affect the activity formed. ~~From the stability of the measured inhibition zone, the~~ The antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram-positive bacteria and ~~are~~ were merely bacteriostatic for Gram-negative bacteria. As gram-~~-~~positive symbiotic bacteria widely know~~s~~ 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 indicate~~s~~ 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.

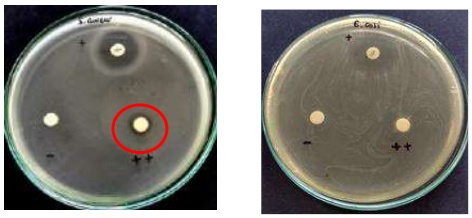

Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

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

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

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

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

Identification of Phenotype and Genotype of 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-

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motile, ~~developing~~ ~~and~~ grow ~~vigorous~~aerobically, negative catalase, and positive carbohydrate testy~~.~~ ~~catalase-negative, and a positive test for carbohydra~~ tes. In general, the selected isolate showed special characteristics possessed by lactic acid bacteria ~~common, the distinguishing proof of chosen segregates appeared particular characteristics of lactic corrosive microscopic organisms~~ (Lactobacillus spp.), ~~s~~ 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~~ The h~~H~~ighest 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 ~~of~~ the bacterial isolate is Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus; Lactobacillus plantarum.~~.~~

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

Sequens of 16S rDNA

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGAT
TTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAG
ATGCTAATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCG
CGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACA
TTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAG
CAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCA
GGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTG
TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTG
CATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGA
AGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAG
ATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCAT
TAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGC
ATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTAGACGTTCCC
TTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCG
CAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGA
TGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCG
AGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGT
AATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACA
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 of 100% of the sequences present in GenBank. The species homology of the tested isolate was *Lactobacillus plantarum*. *Lactobacillus plantarum* strains separated from dairy items appeared solid antimicrobial action against the pointers strains of *Staphylococcus aureus*, *Salmonella spp*, and *Escherichia coli* (Hu ~~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 L~~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* was ~~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 publication~~s~~ funding ~~fund~~.

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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, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer properties of *Turbinaria conoides* (J. Agardh). Clin Phytosci. 3:5

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

Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, Chuang YC, Lu YC, and Tang HJ, 2019. Antimicrobial Activity of *Lactobacillus* Species Against Carbapenem-Resistant *Enterobacteriaceae*. *Front. Microbiol.* 10:789

Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015. Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. ~~Pubmed.~~Publish in Anaerobe-, ISSN 1075-9964; Vol. 34; pp. 53 – 58.

Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological mechanisms and Applications. Mar Drugs. (2016) Apr; 14(4): 81.

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

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

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

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

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

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

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

Irma E~~sthela S~~oria-M~~ercado~~, ~~Luis Jesús Villarreal~~-Gómez LJV, ~~Graciela Guerra~~-Rivas GG, and ~~Nahara E. Ayala~~-Sánchez NEA, 2011. Bioactive Compounds from Bacteria Associated to Marine in Algae. Biotechnology: Molecular Studies and Novel Applications for Improved Quality of Human L. BoD – Books on Demand, 2012 (~~25237~~)

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 Sci. 2016 April; 7(2): (B) 395 – 405. India.

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

Li D, Ni K, Pang H, Wang Y, Cai Y, Jin Q. 2015. Identification and Antimicrobial Activity Detection of Lactic Acid Bacteria Isolated from Corn Stover Silage. Asian-Australas J Anim Sci. 2015 May; 28(5): 620–631.

~~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 of *Lactobacillus* Species Against Carbapenem-Resistant *Enterobacteriaceae*. *Front. Microbiol.* 10:789~~

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

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

Mezaini **A**, Chihib N E, Bouras A D, Arroume N N, Hornez J P. 2009. Antibacterial Activity of Some Lactic Acid Bacteria Isolated from an Algerian Dairy Product. Journal of Environmental and Public Health. Volume 2009.

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

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

~~Noora B, Saeid TJ, Hadi BP, Fabio V, 2019. Metabolites from Marine Microorganisms, Micro, and Macroalgae: Immense Scope for Pharmacology. Mar Drugs. 2019 Aug; 17(8): 464.~~ O'Donnell, 1993. Fusarium and its Near Relatives. National Center for Agriculture Utilization Research, USDA, ARS, 1815 N. University Street, Peoria, Illinois, 61604, USA.

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

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.

Smaoui S, Elleuch L, Bejar W, Karray-Rebai I, Ayadi I, Jaouadi B, Mathieu F, Chouayekh H, Bejar S, Mellouli L. 2010. Inhibition of fungi and gram-negative bacteria by bacteriocin BacTN635 produced by Lactobacillus plantarum sp. TN635.Appl Biochem Biotechnol. 2010 Oct: 162(4):1132-46.

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

White TJ, Bruns T, Lee S, 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.

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BIODIVERSITAS
Volume 22, Number 1, January 2021
Pages: 373-377

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220145

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

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

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

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

INTRODUCTION

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

In later decades, strides microbiological procedures have altogether made a difference in build-up phylogenetic affiliations of seaweed-related epi-bacterial communities and endophytes. Be that as it may, there is inadequately prove that utilitarian connections for seaweed-bacterial intuitive can be built up and well caught on. Epiphytic bacterial communities are rapid colonizers of the ocean growth surface, some of the time versatile and able to quickly metabolize algal exudates (Singh and Reddy 2014). It has traditionally been used for children's fever, as a fertilizer, repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta and Abu-Ghannam 2011). Seaweeds can secrete secondary metabolites with antibacterial properties (Shannon and Abu-Ghannam 2016). The symbiotic mutualism occurs as algae provide essential sites and nutrients, while the bacteria encourage growth and protect the algal surface against symbiont bacteria isolates as algae have abundant antimicrobial activity. The existence of the bacteria is suspected as a result of infections acquired from the community (Arumugama et al. 2017). *Turbinaria conoides* is a tropical marine alga widely distributed in coastal waters in Asia.

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

MATERIALS AND METHODS

Sampling

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

Isolation of symbiont bacteria producing antibacterial compounds

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

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

After the extraction process, 1 mL of refresh samples were diluted in a 9 mL of sterile nutrient broth to make 10^{-1} dilution. This process was continued to achieve 10^{-5} dilutionEach 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.

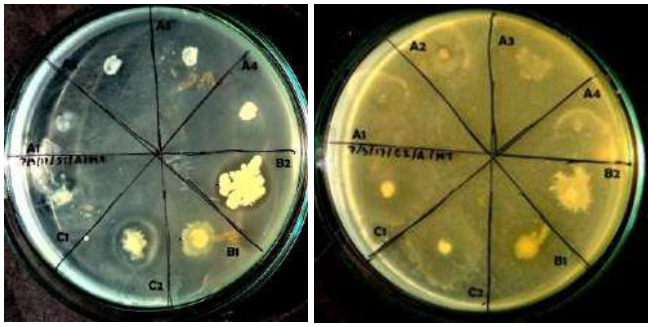
Tabel 1. Macroscopic forms of bacterial colonies.

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

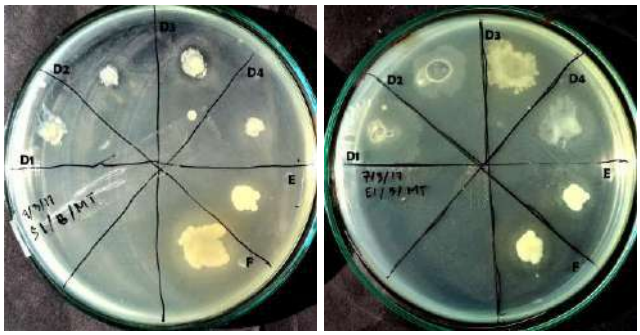
Table 2. Macroscopic form of the isolates on slant agar

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



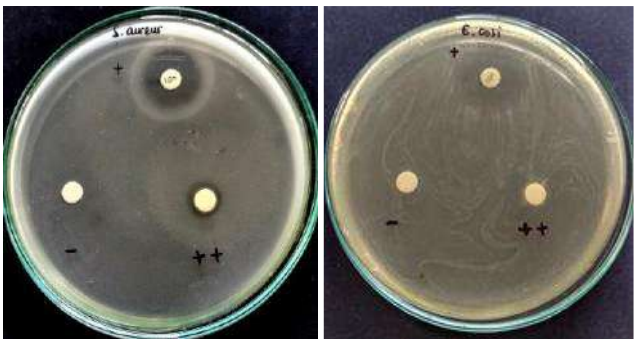
A B

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)



A 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)



A 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 know contain bacteriocins (Mezaini et al. 2009; Li et al. 2015) bacteriocins from Gram-positive bacteria are generally not effective against Gram-negative bacteria (Smaoui et al. 2010). Paper disc with supernatant applied to a Gram-positive bacterial plate indicate a stable clear zone even after a 48-hour incubation period. While against the Gram-negative bacteria, the presence of inhibitory activity appeared around the disc paper, but it was gradually turbulent before the incubation period reaches 24 hours. The antibacterial compounds produced by 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

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAG
TGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTT
GGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCA
CCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGG
GAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAA
CATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGC
ATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGG
CGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAA
CGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTG
AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACAT
ACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGA
GTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC
ATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTC

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

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

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

ACKNOWLEDGEMENTS

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

REFERENCES

Andrea GZ, Miguel A. Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara. 2019. The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. Antioxid (Basel) 8 (9): 406.

Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer properties of *Turbinaria conoides* (J. Agardh). Clin Phytosci 3: 5. DOI: 10.1186/s40816-017-0042-y.

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

Baquero F, Levin BR. 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. Nat Rev Microbiol. DOI: 10.1038/s41579-020-00443-1.

Barzkar N, Jahromi ST, Poorsaheli HB, Vianello F. 2019. Metabolites from marine microorganisms, micro, and macroalgae: immense scope for pharmacology. Mar Drugs 17 (8): 464. DOI: 10.3390/md17080464.

Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, Chuang YC, Lu YC, Tang HJ. 2019. Antimicrobial activity of *Lactobacillus* species against carbapenem-resistant Enterobacteriaceae. Front Microbiol 10: 789. DOI: 10.3389/fmicb.2019.00789.

Davoodabadi A, Soltan Dallal MM, Rahimi Foroushani A, Douraghi M, Sharifi Yazdi MK, Amin Harati F. 2015. Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Anaerobe 34: 53-58.

Grela E, Kozłowska J, Grabowiecka. 2018. A. Current methodology of MTT assay in bacteria-a review. Acta Histochem 120 (4): 303-311

Gupta S, Abu-Ghannam N. 2011/Bioactive potential and possible health effects of edible brown seaweeds. Trends Food Sci Technol 22: 315-326.

Hu CH, Ren LQ, Zhou Y, Ye BC. 2019. Characterization of antimicrobial activity of three *Lactobacillus plantarum* strains isolated from Chinese traditional dairy food. Food Sci Nutr 7 (6): 1997-2005. DOI: 10.1002/fsn3.1025.

Kapoor G, Saigal S, Elongavan A. 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. J Anaesthesiol Clin Pharmacol 33 (3): 300-305.

Li D, Ni K, Pang H, Wang Y, Cai Y, Jin Q. 2015. Identification and antimicrobial activity detection of lactic acid bacteria isolated from corn stover silage. Asian-Australas J Anim Sci 28 (5): 620-631.

Manisha DM, Shyamapada M. 2011. Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed1 (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 Holomarph Mititic, Meiotic and Pleomorpic 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 Pathogenesis115: 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.

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

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ABSTRACT

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

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

INTRODUCTION

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

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

MATERIALS AND METHODS

Procedures

Sampling

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

Isolation of Symbiont Bacteria Producing Antibacterial Compounds

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

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

Selection of Symbiont Bacteria Isolates Antagonistically against Pathogenic Bacteria

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

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

Antibacterial Potential Testing of Symbiont Bacterial Isolate by Paper Disc Diffusion

Testing the supernatant of symbiont bacteria for inhibitory growth of *E.coli* and *S.aureus* was performed by the agar diffusion method (Hudzicki, 2009)). Supernatant was obtained by separating the filtrate and supernatant bycentrifuge for 1 hour (25 ^{o}C and 3000 rpm). Paper discs containing supernatant 40 µL and the negative control nutrient broth 40 µL were left for 1 hour to reduce the water excess, and positive control chloramphenicol 0.01 mg/mL, 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. 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 to assess bioactivity as sensitive, resistant, or intermediate, in each case the resistance zone where shows no colonies growth was measured by using ruler to the nearest mm.

Identification of Phenotype and Genotype of Symbiont Bacteria

General bacterial identification (Lay, 1994 and identification keys from Cowan and Steel (1993)) followed colony characteristic observations on liquid medium and solid medium, observing cell morphology (gram staining, spore staining, and Ziehl-Neelsen staining), and 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^{o}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 *Turbinaria conoides*. Determination of the type of bacteria was performed based on. 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 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

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 epibionts, while the other 8 came from the algal tissue. The results of identification of colonies grown on mixed cultures can be seen in Table 1. and identification of isolates isolated into slant agar can be seen in Table 2.

Tabel 1. Macroscopic forms of bacterial colonies

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

Information:
*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
** The code of isolates ([2]), ([4]), ([5]), ([3]) states isolates obtained from the dilution
*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient sequence and the number code representing isolates in the order of the first, second, and so on according 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. 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

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 (Dwidjoseputro, 1981).

The Selection Results Symbiont Bacteria Producing Antibacterial Compounds

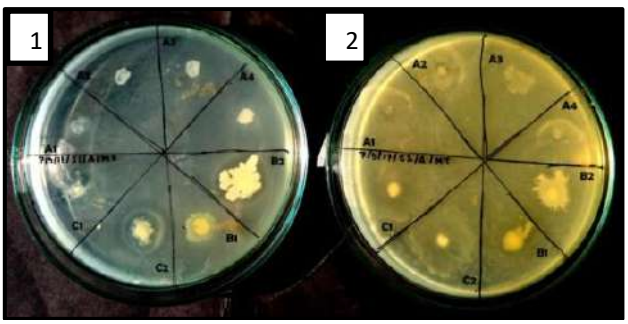

Figure 1. Symbiont bacterial isolates (A1, A2, A3, A4, B1, B2, C1, C2) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

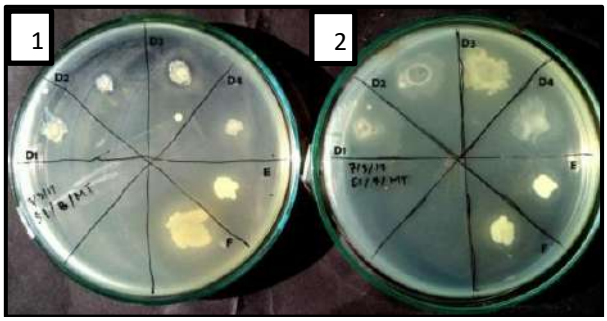

Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (1) and *E.coli* (2)

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

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

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

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

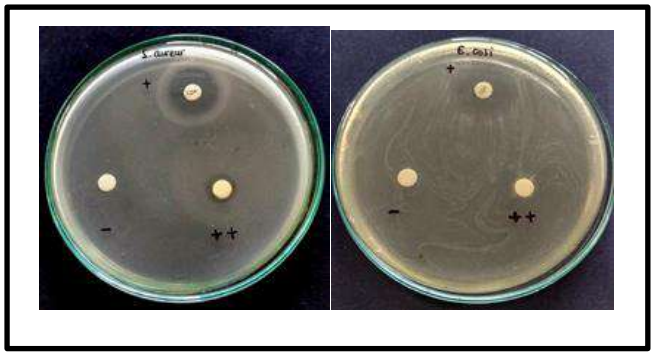

Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

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

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

The area of the symptomatic supernatant inhibition zone of *S.aureus* was 6.7 mm. According to Edrada (1998) in Kusumadewi (2004) 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 (Gudbjarnason 1999 in Nofiani, 2005). Seawater contains an active inhibitor agent for Gram positive bacteria Okami (1982) in Nofiani (2005)

Identification of Phenotype and Genotype of Symbiont Bacteria

Based on phenotypic identification results through cell staining and biochemical testing, 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 (Desniar 2012 in Saskia, 2014).

*Lactobacillus plantarum*_100%

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACAT
TTGAGTGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCG
CATAACAACTTGGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATG
GTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAA
CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTC
GGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTA
ACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT
AAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTC
CATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTC
GAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCT
TCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAGAGATTA
GACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG
AGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG
ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGAGTAAG
CTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC
ATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTC

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*.

CONCLUSION

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

REFERENCES

Cowan ST and Steel KJ. 1993. *Manual for the Identification of Medical Bacteria*. 3rd Edition. University of Cambridge. UK.Dwidjoseputro, D. 1981. *Dasar-dasar Mikrobiologi*. Cetakan ke-5. Djambatan, 1981: Jakarta.

Hudzicki. 2009. *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol, ASM Microbelibrary*. American Society for Microbiology. http://www. microbelibary.org/component/resource/laboratory-test/3189-kirby-bauerdisk-diffusion-susceptibility-test-protocol., (7/04/2014)

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

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

Lay BW. 1994. *Analisis Mikroba di Laboratorium*. PT. Raja Grafindo Persada: Jakarta

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

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

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

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

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

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

Sulistijowati R and 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.

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 Aplications, Academic Press, Inc., New York.

BIODIVERSITAS
Volume 22, Number 1, January 2021
Pages: 373-378

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220145

FILE 2. BUKTI ACCEPTED / TANGGAL 26 DESEMBER 2020

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

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

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

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

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

INTRODUCTION

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

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

MATERIALS AND METHODS

Procedures

Sampling

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

Isolation of symbiont bacteria producing antibacterial compounds

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

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

Selection of symbiont bacteria isolates antagonistically against pathogenic bacteria

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

Antibacterial potential testing of symbiont bacterial isolate by paper disc diffusion

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

Identification of phenotype and genotype of symbiont bacteria

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

RESULTS AND DISCUSSION

The result of symbiont bacteria isolation

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

The selection results symbiont bacteria producing antibacterial compounds

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

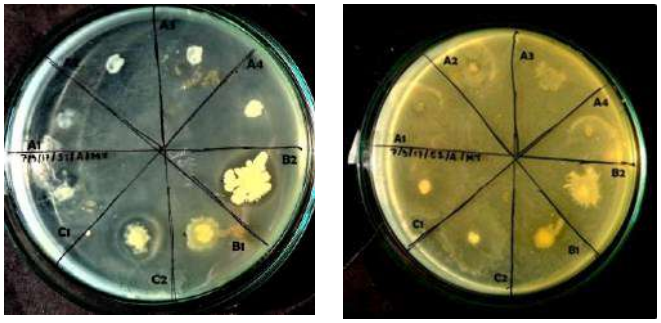
Tabel 1. Macroscopic forms of bacterial colonies.

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

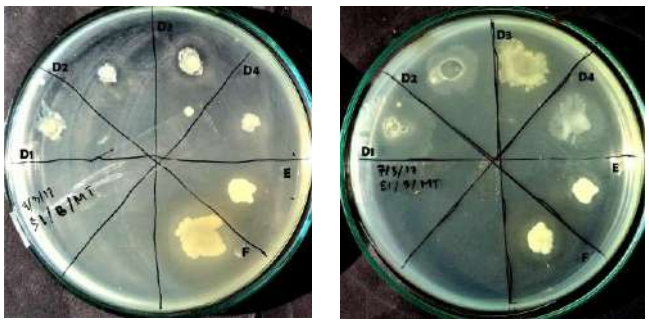
Table 2. Macroscopic form of the isolates on slant agar

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



A **B**

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)



A **B**

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

Isolates with code TUD4-C2-2 had the best inhibition zone. Bacterial isolates derived from algal tissue showed better inhibition than isolates derived from epibionts. The inhibitory zone and diameter measurement results against *S. aureus* and *E.coli* can be seen in Figure 3 and Table 3. Positive controls showed 16.8 mm inhibition zone against *S. aureus* and 13 8 mm inhibition zone against *E. coli*. Chloramphenicol with a concentration of 0.03 mg on a paper disc is highly active if its inhibition zone is more than 18 mm (Mounyr et al., 2016), while the dose of chloramphenicol (positive control) used was less than 0.01 mg, so it can be said that bacteria test was found to be sensitive to the positive control. Negative control (NB without symbiotic bacterial inoculation) indicates the absence of activity or inhibition zone, so it can be ascertained that supernatant does not affect the activity formed. The antibacterial properties of the supernatant produced by the symbiotic bacteria act as inhibitors against Gram-positive bacteria and were merely bacteriostatic for Gram-negative bacteria. As gram-positive symbiotic bacteria widely know contain bacteriocins (Mezaini A et al, 2009 and Li D. Et al, 2015) bacteriocins from Gram-

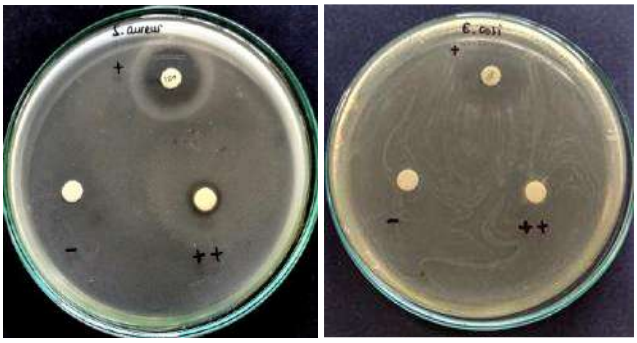
positive bacteria are generally not effective against Gram-negative bacteria (Smaoui et al, 2010). Paper disc with supernatant applied to a Gram-positive bacterial plate indicate a stable clear zone even after a 48-hour incubation period. While against the Gram-negative bacteria, the presence of inhibitory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

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

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

Identification of phenotype and genotype of symbiont bacteria

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



A 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

Sequens of 16S rDNA

GCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAACTCTGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAG
TGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAACAACTT
GGACCGCATGGTCCGAGCTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCA
CCATGGCAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGG
GAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAA
CATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGC
ATCGGAAACTGGGAAACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAGAACACCAGTGG
CGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAA
CGATGAATGCTAAGTGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTACGGCCGCAAGGCTG
AAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACAT
ACTATGCAAATCTAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG

GTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAA
GGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAACGAGTTGCGAACTCGCGAGA
GTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGC
ATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGTC

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

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

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

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

ACKNOWLEDGEMENTS

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

REFERENCES

Andrea GZ, Miguel A. Prieto L, Cecilia J-Lopez, Juan C. Mejuto, Jesus S-Gandara, 2019. The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. Antioxid (Basel) 8(9): 406.

Arumugama P, Kavipriya R, Murugan M, Ramar M, Kamalakannan S, Murugan K 2017. Antibacterial, antioxidant, and anticancer properties of *Turbinaria conoides* (J. Agardh). Clin Phytosci 3: 5

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

Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, Chuang YC, Lu YC, and Tang HJ, 2019. Antimicrobial Activity of *Lactobacillus* Species Against Carbapenem-Resistant *Enterobacteriaceae*. Front Microbiol 10:789

Davoodabadi, Abolfazl; Soltan D, Mohammad M; Rahimi F, Abbas; D, Masoumeh; Sharifi Y, Mohammad K; Amin H, Farzaneh, 2015. Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Publish in Anaerobe 34: 53-58.

Emer Shannon and Nissreen Abu-Ghannam, 2016. Antibacterial derivatives of marine Algae: An overview of pharmacological mechanisms and Applications. Mar Drugs 14 (4): 81.

Erin R sanders. 2012. Aseptic Laboratory Techniques: Plating Methods. J Vis Exp. 63: 3064

Baquero F, Levin BR.. 2020. Proximate and ultimate causes of the bactericidal action of antibiotics. Nat Rev Microbiol (2020)

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

Grela E, Kozłowska J, Grabowiecka, 2018. A. Current methodology of MTT assay in bacteria-a review. Acta Histochem 120 (4): 303-311

Gupta S, Abu-Ghannam N. Bioactive potential and possible health effects of edible brown seaweeds. Trends Food Sci Technol 22: 315-326.

Hu CH, Ren LQ, Zhou Y, Ye BC. 2019. Characterization of antimicrobial activity of three Lactobacillus plantarum strains isolated from Chinese traditional dairy food. Food science & Nutrition 7 (6): 1997–2005. DOI: 10.1002/fsn3.1025

Irma ESM, Gómez LJV, Rivas GG, and Sánchez NEA, 2011. Bioactive Compounds from Bacteria Associated to Marine in Algae. Biotechnology. Molecular Studies and Novel Applications for Improved Quality of Human Life, Reda Helmy Sammour, IntechOpen. DOI: 10.5772/27842, https://www.intechopen.com/books/biotechnology-molecular-studies-and-novel-applications-for-improved-quality-of-human-life/bioactive-compounds-from-bacteria-associated-to-marine-algae

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

Garima K, Saurabh S, Ashok E. 2017. Action and resistance mechanisms of antibiotics: A guide for clinicians. J Anaesthesiol Clin Pharmacol. 33 (3): 300-305.

Li D, Ni K, Pang H, Wang Y, Cai Y, Jin Q. 2015. Identification and Antimicrobial Activity Detection of Lactic Acid Bacteria Isolated from Corn Stover Silage. Asian-Australas J Anim Sci. 2015 May; 28(5): 620–631.

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

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

Mezaini **A**, Chihib N E, Bouras A D, Arroume N N, Hornez J P. 2009. Antibacterial Activity of Some Lactic Acid Bacteria Isolated from an Algerian Dairy Product. Journal of Environmental and Public Health. Volume 2009.

Monte, J., Abreu, A. C., Borges, A., Simões, L. C., & Simões, M. (2014). Antimicrobial Activity of Selected Phytochemicals against

Escherichia coli and Staphylococcus aureus and Their Biofilms. *Pathogens (Basel, Switzerland)*, *3*(2), 473–498.

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

O'Donnell. 1993. Fusarium and its Near Relatives. National Center for Agriculture Utilization Research, USDA, ARS, 1815 N. University Street, Peoria, Illinois, 61604, USA.

Phumudzo T, Ronald N, Khayalethu N, Fhatuwani M. 2013. Bacterial species identification getting easier. Afr J Biotechnol 12 (41): 5975-5982

Singh RP, Reddy CRK. 2014. Seaweed–microbial interactions: key functions of seaweed-associated bacteria. FEMS Microbiol. Ecol 88 (2): 213-230.

Smaoui S, Elleuch L, Bejar W, Karray-Rebai I, Ayadi I, Jaouadi B, Mathieu F, Chouayekh H, Bejar S, Mellouli L. 2010. Inhibition of fungi and gram-negative bacteria by bacteriocin BacTN635 produced by Lactobacillus plantarum sp. TN635. Appl Biochem Biotechnol. 162 (4): 1132-46.

Wang L, Zhang H, Rehman M.U, Khalid Mehmood K, Jiang X, Iqbal M, Tong X, Gao X, Li J,2018. Antibacterial activity of *Lactobacillus plantarum* isolated from Tibetan yaks. J Microbial Pathogenesis115: 293-298.

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

FILE 3. PERTANYAAN/PERINTAH PERBAIKAN DARI PARA REVIEWER JURNAL

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

Editor/ Reviewer	Date	Reviewer Suggestion	Author response
Editor	**9-10-2020**	Dear author, Thank you very much for your manuscript submission. Unfortunately, your manuscript does not meet our requirements: - At least, to published in the Biodiversitas journal, you need to compose a minimum of 20 references which 80% of international scientific journals published in the last 10 years (2010-2020), and maximum 10% references in the local language (not English). And please write the references based on the author's guidelines. -This manuscript is too brief to be published in the Biodiversitas journal. At least, you need to compose a 2000 words article from the introduction to a conclusion (table and figure are excluded). Kindly check and correct accordingly Thank you	The number of references has been added to 27 libraries which originally amounted to 13 libraries. The number of 27 libraries has exceeded the minimum limit of 20 libraries. Literature composition consists of international journals of science for the last 10 years (2010-2020) as many as 23 libraries or 85% and 0% of local languages meet a minimum of 10% of local languages (not English) The number of words has been added to 2208 words outside Tables and Figures that have exceeded the minimum limit of 2000 words The addition of these words is done by developing a discussion using reference references.
Reviewer B:	**9-11-2020**	Dear authors of the manuscript: Antibacterial potential of symbiont bacteria of brown algae (Turbinaria conoides) obtained from Indonesian waters. The manuscript focus on symbionts from this macroalga, which may present antibacterial properties. It was with pleasure that I read the work. The study seems relevant, interesting, and important. Nowadays the research of new antimicrobial compounds is crucial and it is known that the sea is a reservoir of this kind of compound. Although I think the outputs selected for the study were	The paper has been improved as requested by adding a discussion of both macroalgae and symbiotic bacteria using libraries from several recent journals

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

		from elsewhere may be important for someone interested in the distribution and diversity of Lactobacillus. 3. Lactobacillus plantarum is well-known for its antibacterial activity, see eg: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6593389/ https://www.sciencedirect.com/science/article/abs/pii/S0882401017314559 https://www.frontiersin.org/articles/10.3389/fmicb.2019.00789/full I think the dicussion and conclusions would benefit from referencing this fact. Should these issues be fixed, I believe the paper will be acceptable for publication. Recommendation: Revisions Required	
Reviewer A:	**11-12-2022**	1. You did not mention direct test in the method but you wrote it your results. 2. You wrote the results but you did not discuss it with other results add some references. 3. You isolated 14 isolates. Were they all from *Lactobacillus*. 4. Write references as per the rule of journal norm there are so many mistakes. 5. Some references are missing in the reference so please check it.	Paper has been improved as follows: 1. In the methods section, it is clear about how to te the results in the Results and discussion section. 2. In the results and discussion section, discussion h including the results of other studies based on ref 10 years (2010 – 2020). 3. The authors obtained 14 isolates of symbiont bac one symbiont with the best inhibitory zone agains coli bacteria to be tested for DNA sequencing and sequencing results as Lactobacillus plantarum. 4. Bibliography writing has been improved according writing a Library (APA). 5. Already fixed missing Library writes,