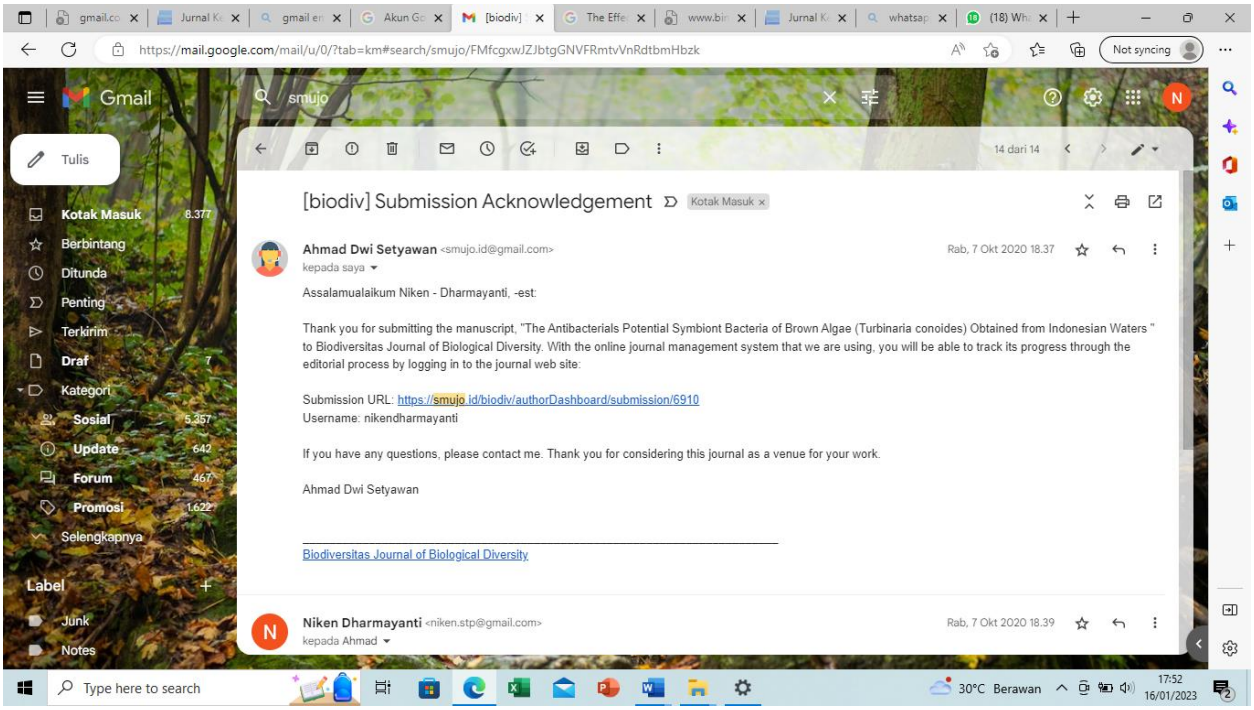
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Assalamualaikum Niken - Dharmayanti, -est:
Thank you for submitting the manuscript, "The Antibacterials Potential Symbiont Bacteria of Brown Algae (Turbinaria conoides) Obtained from Indonesian Waters " to Biodiversitas Journal of Biological Diversity. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:
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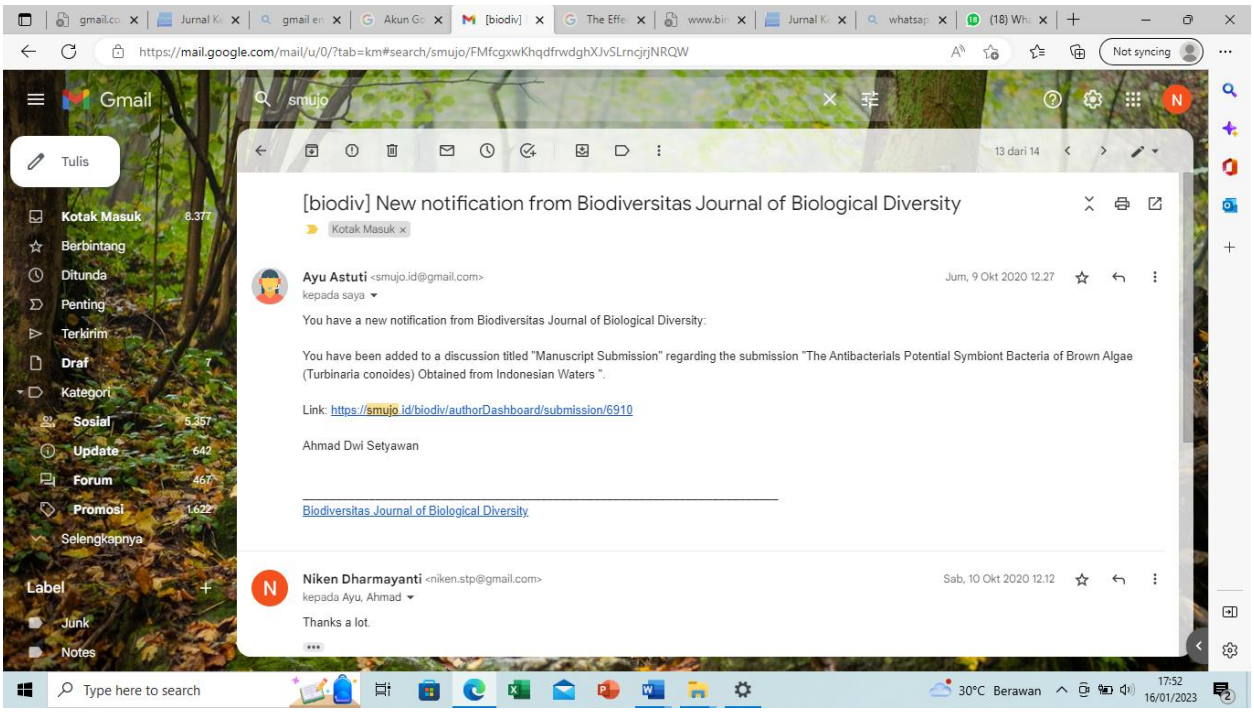
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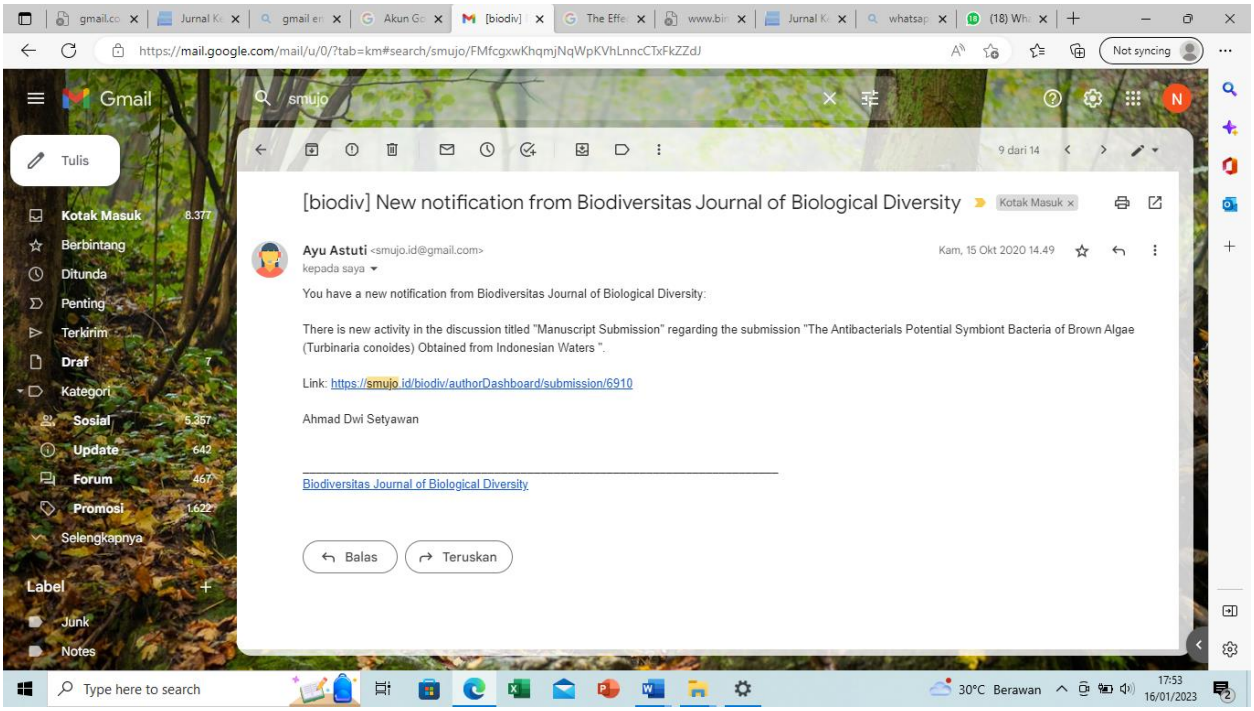
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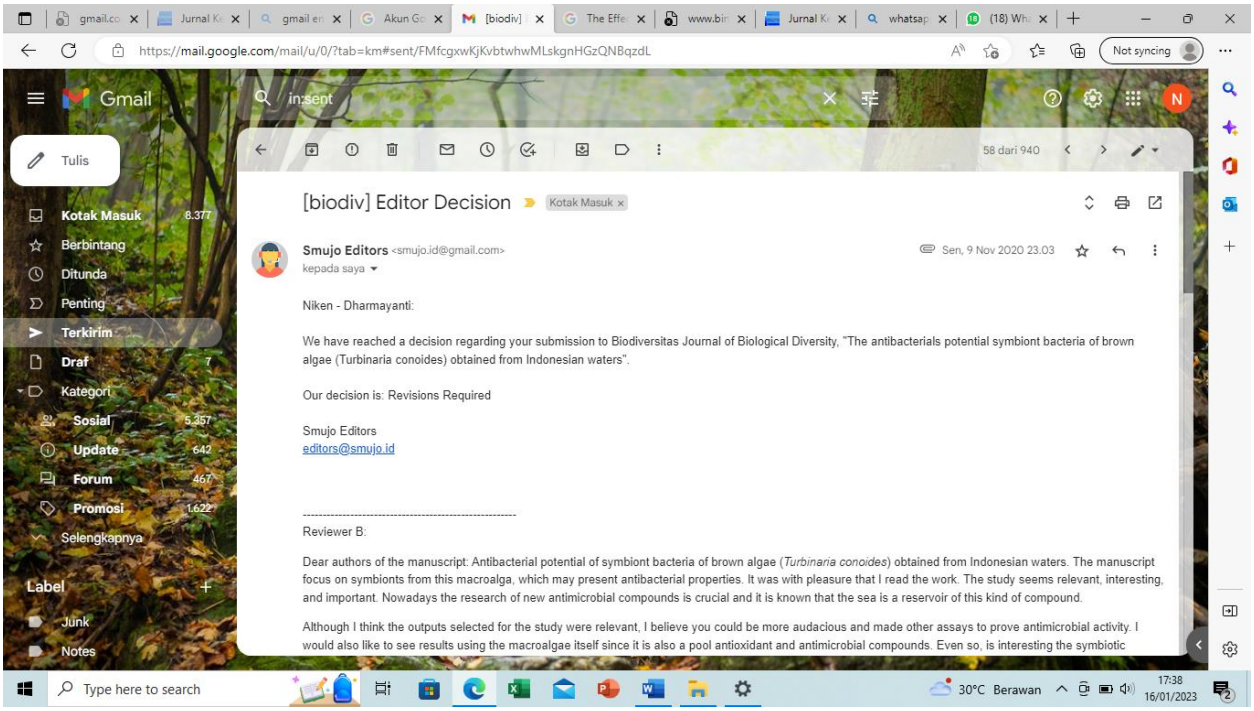
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Niken - Dharmayanti:
We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "The antibacterials potential symbiont bacteria of brown algae (Turbinaria conoides) obtained from Indonesian waters".
Our decision is: Revisions Required
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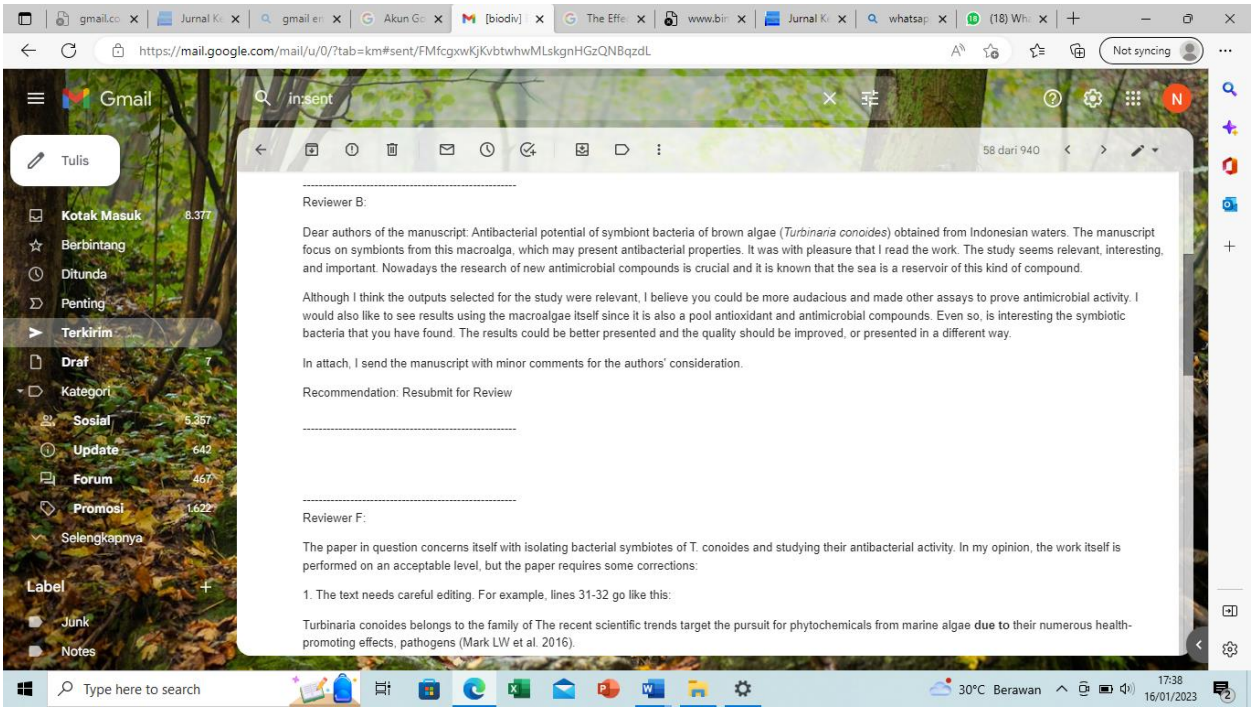
editors@smujo.id
Reviewer B:
Dear authors of the manuscript: Antibacterial potential of symbiont bacteria of brown algae (Turbinaria conoides) obtained from Indonesian waters. The manuscript focus on symbionts from this macroalga, which may present antibacterial properties. It was with pleasure that 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 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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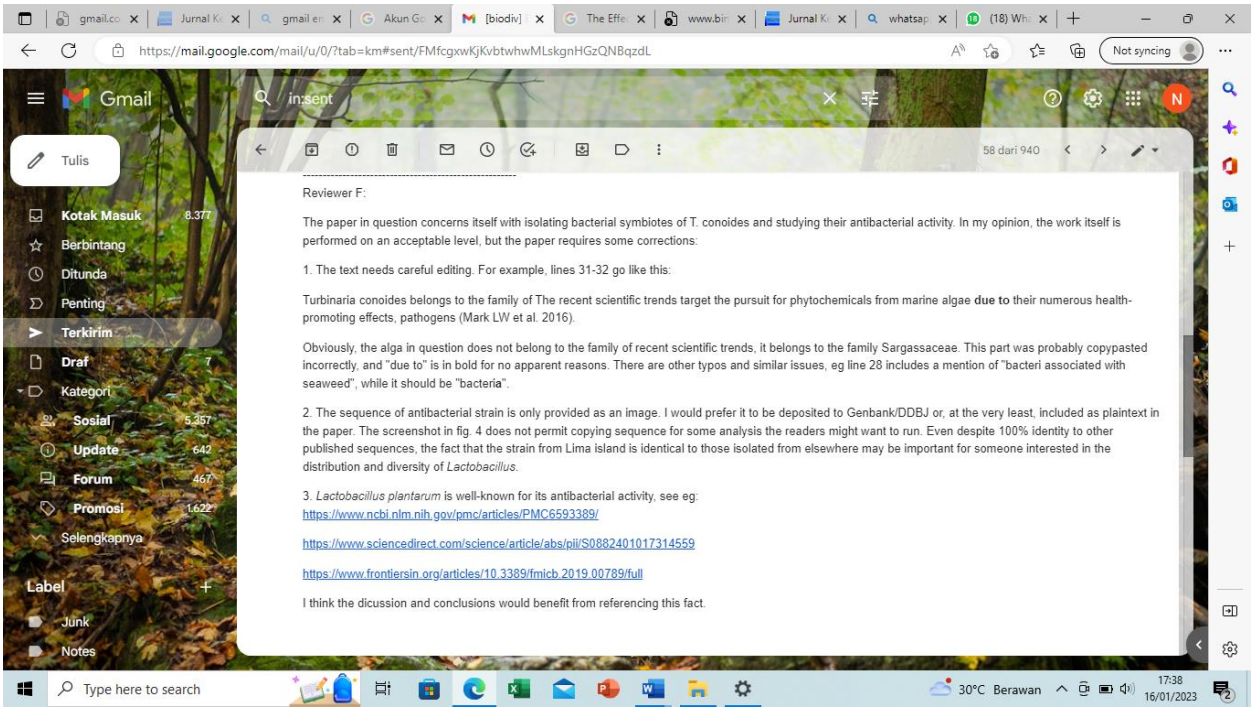
In attach, I send the manuscript with minor comments for the authors' consideration.
Recommendation: Resubmit for Review
Reviewer F:
The paper in question concerns itself with isolating bacterial symbiotes of T. conoides and studying their antibacterial activity. In my opinion, the work itself is performed 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).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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 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:
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Antibacterial~~s~~ potential of symbiont bacteria of brown algae (*Turbinaria conoides*) obtained from Indonesian waters

Abstract. Brown seaweed~~s~~ ha~~ves~~ the potential to produce bioactive compounds. It has been shown that the bacteria associated with seaweed~~s~~ 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~~found, 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 ~~bacteri~~bacteria 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, ~~repellant~~repellent, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer (Gupta et al. 2011) *Turbinaria conoides* belongs to the family of t~~T~~he 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). T~~t~~he 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.

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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 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.

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.

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/)

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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

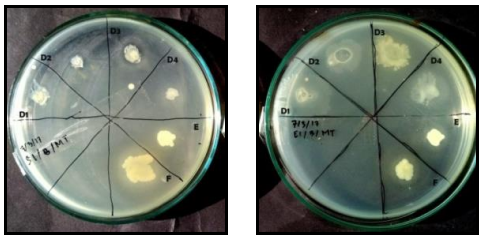
Code of isolates	Solid medium	
	Shape	Color
TUL[2]-A1-2	Spread	Milky white
TUL[2]-A2-2	Spread	Milky white
TUL[2]-A3-2	Spread	Milky white
TUL[2]-A4-2	Spread	Milky white
TUL[2]-B1-2	Rhizoidal	Cloudy white
TUL[2]-B2-2	Rhizoidal	Cloudy white
TUD[4]-C1-2	Spread	Milky white
TUD[4]-C2-2	Spread	Milky white
TUD[2]-D1-2	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

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)

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

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

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

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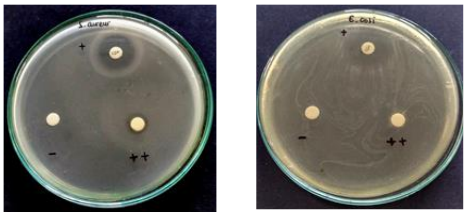

Figure 3. Results of antibiotic susceptibility test against *S.aureus* and *E.coli*

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 test~~sing~~, 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%

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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.

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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

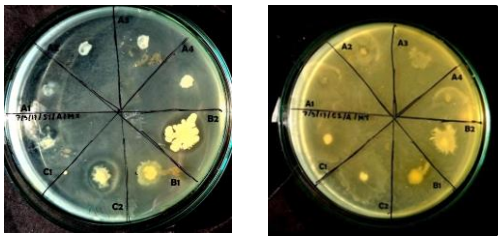
Note:
*The code of isolates TUL/TUD states the isolates originating from the outer/inner algae
** The code of isolates ([2]), ([4]), ([5]), ([3]) states isolates obtained from the dilution
*** The code of isolates A1 and so on, B1 and C1 so on, D1 so on, E, F, the letter codes denote the observed gradient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

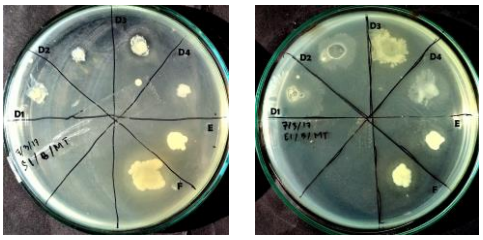
~~Bacteria is isolated into a solid medium, then there is a group commonly referred to as a colony. The colony's shape is different for each species and it is characteristic of a particular species (Erin RS 2012).~~ Bacteria were isolated in a solid medium and the size of the colony was different for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

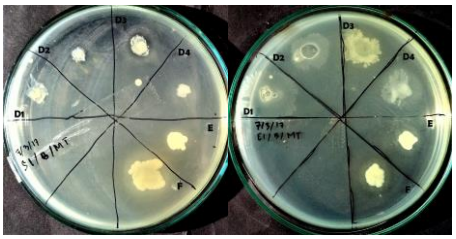


Figure 2. Symbiont bacterial isolates (D1, D2, D3, D4, E, F) on a direct challenge test to *S.aureus* (A) and *E.coli* (B)

~~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 ~~vigorously~~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
TCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTG
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 l~~actobacillus ~~–~~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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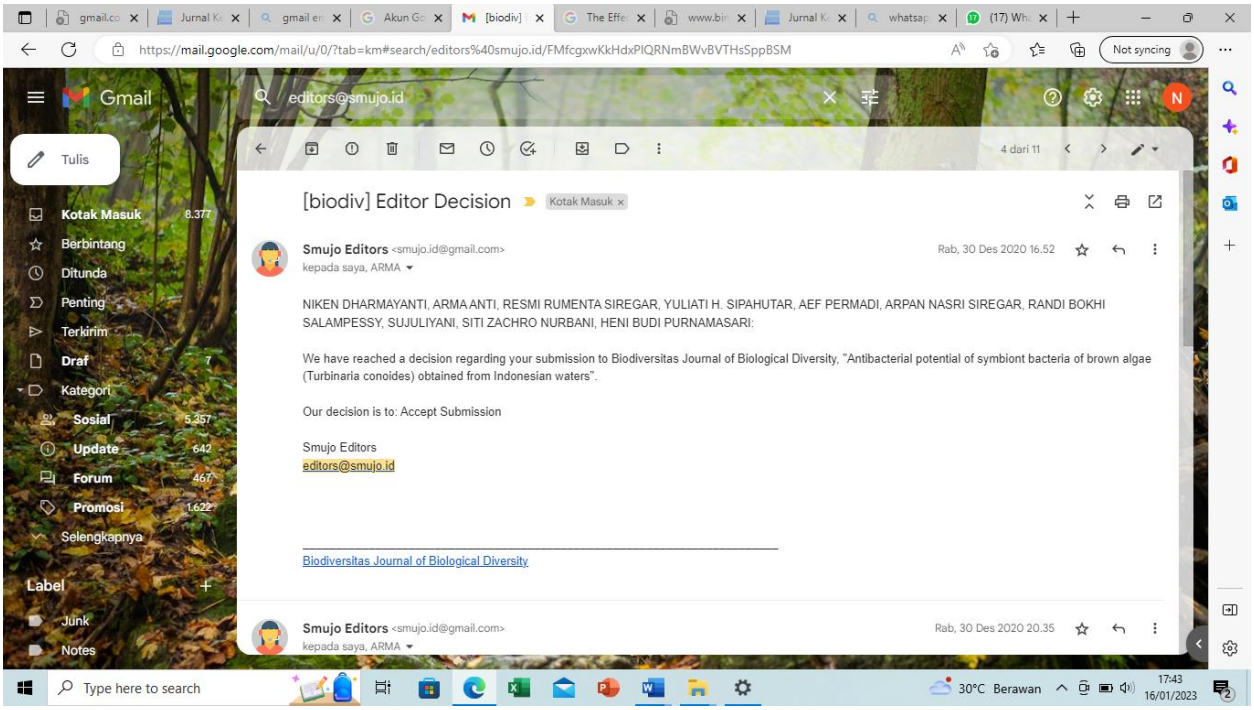
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