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Antimicrobial Activity of Endophyte Bacteria Isolated from Seaweed (*Padina* sp.) on The Growth of Bacteria *Escherichia coli* and *Staphylococcus aureus*

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Abstract

Padina sp. seaweed has the potential to become one of the producers of bioactive compounds. Padina sp. one of the seaweeds from Chlorophyta which has a lot of potential in it and still little has been explored. This study aims to determine the antimicrobial activity of endophytic bacteria from Padina sp.seaweed endophytic bacteria against Escherichia coli and Staphylococcus aureus. The method used in this research is descriptive qualitative. The results of the isolation of endophytic microbes Padina sp. The seaweed obtained was seven isolates DP1, DP2, DP3, DP4, AP1, AP2, and AP3. The results of the antimicrobial activity test showed that the seven endophytic bacterial isolates had potential as antimicrobials. Incubation time of 24 hours showed that the diameter of the inhibition zone formed was 9.75 – 11.55 mm for S.aureus and 7.15 – 10.0 mm for bacteria. E. coli. AP3 and DP1 isolates had wider zones, presumably because their antibacterial properties were higher than the other isolates.
Keywords: *Antimicrobial, Padina sp., Seaweed*

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INTRODUCTION

Seaweed is one of the traditional medicines that was first used by the Chinese empire. Bioactive compounds produced by seaweed can be used by humans as medicinal ingredients. Several seaweeds have been reported to be used as antimicrobial, antioxidant, antibiotic, anti-inflammatory, antiproliferative, anticoagulant, and antitumor (Nawaly and Susanto, 2008). One of the potential seaweeds is *Padina* sp as a producer of bioactive compounds. *Padina* sp. is one of seaweeds species from Chlorophyta which has a lot of potential in it and is little explored. Research by Dzeha et al., (2016); Juliasih (2022) found that *Padina* sp produces a triterpenoid compound, namely clionasterol. The obstacle in investigating and exploiting bioactive compounds from seaweed is the limited production in nature. The solution that can be done for the analysis of bioactive compounds from seaweed is endophytic bacteria.

Seaweed is able to associate in deep tissues, one of which is with endophytic bacteria which produce bioactive compounds similar to their host. The association between seaweed and bacteria has been studied for a long time, the interactions that occur between bacteria and seaweed have been carried out for the last 40 years. The potential of seaweed

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endophytic bacteria as producers of bioactive compounds has long been studied and has various potentials such as antimicrobial, antibiotic and antifouling. The study concluded that the bacterial isolates that have the most potential to produce antibacterial compounds were obtained from the species *Padina* sp seaweed (Hollants et al., 2015; Janakidevi et al., 2018; Rozirwan et al., 2015); (Azhar, 2017).

According to Prihatingtyas (2018) Endophytic bacteria are one of the many alternatives that produce antibacterial compounds. The existence of microbes in plants has the possibility of microbes producing bioactive compounds that are uniform to those contained in their parent plants. This research aims to isolate and identify endophytic microbes that have antibacterial potential. Endophytic bacteria are microorganisms that have microscopic sizes that live in the tissues of plant leaves, roots, fruits, and stems. Endophytic bacteria share a mutualism symbiosis with their parent plants with their endophytic bacteria which use nutirent rather than the products of plant metabolism in order to survive. And Bryn (2003) also adding that endophytic bacteria can share beneficial things for the plants they live in, for example, keeping plants fighting herbivores, insects, or pathogenic tissues and can stimulate plant development.

The pathogenic microbial *Escherichia coli* is a microbe that has facultative an-aerobic characteristics and has types of fermentation metabolism and respiration growth more frequently under an-aerobic conditions, but some *E. coli* can live well in aerobic conditions (Meng and Schroeder, 2015). A good temperature for the growth of *E. coli* is 36 °C in a medium containing 1% kpeptone as a source of nitrogen and carbon. The *E. coli* microbe generally has a length of 2.1 µm and a width of 1.0-1.6 µm with the formation of bacilli cells (Melliawati, 2019; Cahyani, 2019). According to (Wijaya, 2019; Fardiaz, 2014) The cell structure consists of E. coli microbes which are composed of their cell walls, plasma membrane, cytoplasm, flagella, nucleus (cell nucleus), and their packages. *Escherichia coli* is a pathogenic microbe that generally causes pathogenicity to food products.

Staphylococcus aureus is a gram-positive microbe that has a spherical shape and a diameter of 0.8-1.3 µm, consists of irregular groups like grapes, facultative an-aerobic, does not form spores, and has no movement. Microbes grow at an optimal temperature of 36ºC but have very good pigment forms at room temperature (21-26ºC). Colonies contain seeds which are full and have a yellowish-gold color, have a round shape, and are shiny. More than 91% of medical isolates produce S. aureus which has a polysaccharide capsule or a thin membrane and has a role in microbial virulence. All degrees of hemolysis are due to S. aureus microbes and rarely from other staphylococcal genera (Jawetz et al., 2015); (Budiarti and Kartika, 2016). *Staphylococcus aureus* is one of the many pathogenic microbes that has links to oxine virulence, invasiveness, and its defense against antibiotics. *Staphylococcus aureus* bacteria can cause various types of infections ranging from mild skin infections, food poisoning to systemic infections (Rahmi et al., 2015; Fardiaz, 2014).

Little and limited information about seaweed from the coast of Tanjung Merah makes it interesting to study and do research. *Padina* sp. is a seaweed that is rich in bioactive compounds, but to prevent excessive exploration, a study was carried out on the endophytic bacteria associated with *Padina* sp. The aim of this research was to determine the antimicrobial activity of endophytic bacteria from *Padina* sp seaweed against *Escherichia coli* and *Staphylococcus aureus* bacteria.

METHOD

This research was conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Manado State University, Tondano, North Sulawesi which took place from 8 February to 11 March 2022. The materials used in this study were distilled water, aluminum foil, 70% ethanol, weighing paper, 5.25% NaOCl solution, Nutrient Agar (NA) medium, Aquades, chloramphenicol antibiotics, Nutrient brooth Medium (NB), Mueller

Hinton Agar (MHA), the test microbes were Escherichia coli, *Staphylococcus aureus*, and samples of *Padina* sp seaweed from Tanjung Merah beach, Matuari District, Bitung City. The tools used were glassware (Pyrex®), autoclave (All American®), petri dish, incubator (Memmert®), tube rack, caliper, Erlenmayer flask, inoculum needle, Laminar Air Flow (Envirco®), spirit lamp, micropipette, aluminum foil, oven, centrifugator, 6mm cork borer, shaker, test tube, analytical balance.

The method used in this research is exploration and experimentation. His exploratory research was to use the method of isolating endophytic microbes in *Padina* sp seaweed found on the coast of Tanjung Merah, Matuari sub-district, Bitung City. Experiments by testing endophytic microbial isolates from *Padina* sp seaweed on pathogenic microbes *E. coli* and *S. aureus*.

Sampling

The sample used was *Padina* sp seaweed obtained from Tanjung Merah beach, Matuari sub-district, Bitung City.

Endophytic Bacteria Isolation

100 grams of *Padina* sp seaweed samples were washed thoroughly with running water then soaked in 70% ethanol solution for 1 minute then soaked in 5.25% sodium hypochlorite for 5 minutes then washed again using 70% ethanol twice rinsed using sterile distilled water for + 1 minute and put it on the surface of the Nutrient Agar (NA) medium which contains nystatin. The medium containing the sample was then incubated in an incubator at room temperature in the dark and observed until bacterial growth appeared. If for 1 day or 24 hours there is no colony growth in the plant sample area, the surface sterilization is declared successful, the microbes are then purified and the purified microbial isolates are then identified morphologically based on the shape and colors of the colonies (Rosana, 2014); (Husni et al., 2014).

Rejuvenation of Pathogenic Bacteria E.Coli and S. Aureus

Take 1 ose one by one the pathogenic microbial colonies, namely E.coli and S.aureus bacteria, then into a petri dish containing NA media using the technique of scratching on the surface so that the NA is slanted and incubated at 35 ° C for 1 day (Soedarto, 2015; Majid et al., 2020).

Endophytic Microbe Testing which has Antibacterial potential

Testing for endophytic microbes was carried out using the well method on E.coli and S.aureus bacteria, one by one the tested bacterial isolates were taken as much as 0.2 milliliters and dripped into the NA medium and then sprinkled evenly using a drygalsky rod. Endophytic bacterial isolates that were one day old in NA agar medium were added with 6 milliliters of 0.85% NaCL. Then put 11 micromyl endophytic microbial suspension into each well punched in the NA medium that had been planted with the test bacteria, incubated for 1 day at 37 ° C, and then observed whether or not a clear zone was formed (Kumala et al., 2010; Majid et al., 2020).

Data analysis technique

Data analysis was carried out by using qualitative descriptive data obtained from the results of endophytic bacteria on Seaweed (*Padina* Sp) which were analyzed descriptively. Quantitative data were obtained from observing the results of antibacterial activity testing on endophytic bacterial isolates, namely the diameter of the inhibition zone.

RESULTS AND DISCUSSION

Endophytic Bacteria Isolation

The parts of the plant to be isolated are the upper end of the leaf, the middle of the leaf, the lower end of the leaf. And in the roots, the parts taken are the top and bottom ends of the roots and the middle part of the roots.

Figure 1. Sections of seaweed obtained from Tanjung Merah beach, Matuari District, Bitung City (a) Leaves, (b) roots, Source: Researcher's Documentation March 7, 2022 Isolation of endophytic bacteria from Seaweed (*Padina* sp) processed as a result of isolation four isolates obtained from the leaves, namely isolates DP1, DP2, DP3, DP4. and three isolates from roots, namely AP1, AP2, and AP3 isolates. The total endophytic bacteria obtained from seaweed were seven isolates.

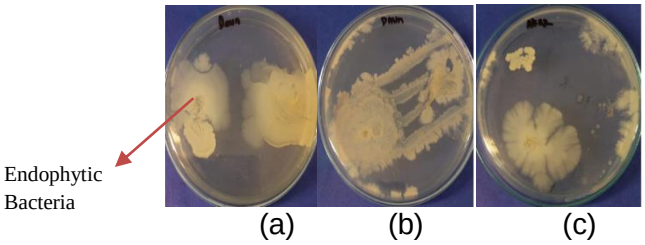

Figure 2. Isolation results, part (a) Upper leaves, (b) Lower leaves, (c) roots.

Colony Morphological Characterization of Endophytic Bacterial Isolates

The morphology of isolated endophytic bacterial colonies which have their own characteristics, therefore the next step is to proceed to the macroscopic observation stage which is covered with the color of the colonies, the shape of the colonies, the edges and the surface of the endophytic bacterial colonies.

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Table 1. Morphological and Physiological Results of Endophytic Bacterial Isolates

Isolate Morphology				Cell Morphology						
Host Bacteria Endophytes	**Code Isolate**	**Form Cell**	**Arrangement Cell**	**Grams**	**Citric**		**Gelatin**	**Starch hydro**	**Sulfide Hydrolysis**	**Genus conjecture**
	DP1	Basil	Single	-	+	-	+	-	-	*Rhizobium*
	DP2	Basil	forming Palisade	+	+	-	+	+	-	*Corynebacterium*
	DP3	Basil	in pairs	-	+	-	+	+	+	*Alcaligenes*
	DP4	Coccus	in pairs	-	+	-	+	+	-	*Paracoccus*
	AP1	Basil	forming V Formation	-	+	+	+	+	+	*Alcaligenes*
	AP2	Coccus	Single	+	+	-	+	+	-	*Stoptylococcus*
	AP3	Coccus	Single	-	+	-	+	+	+	*Proteus*

Bacterial Rejuvenation Test

Take 1 ose of pathogenic bacterial colonies, namely E.coli bacteria and 1 ose of S.aureus colonies into a tube containing NB media, then stir until cloudy and incubate at 36 ° C for 24 hours. 10^6 CFU/ml then incubated for 24 hours (Ningsih, 2013; Rahayu et al., 2018).

Isolate Bakteri Endofit Mikroskopis

Total endophytic bacteria obtained from Seaweed (*Padina* sp) as a result of the isolation results obtained four isolates from the leaves, namely four isolates which were gram negative. And three isolates from roots, namely three isolates, namely the second is a gran negative and o ne isolate which is a gran positive. The total endophytic bacteria obtained from seaweed were seven isolates.

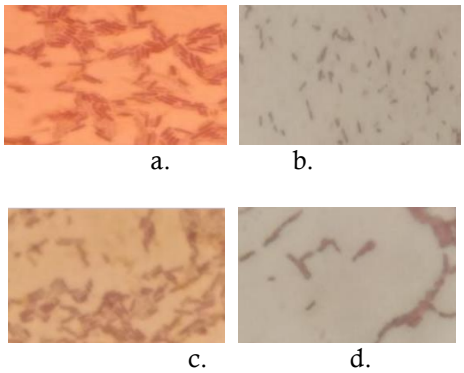

Figure 3. Morphology of leaf isolates (a) DP1, (b) DP2, (c) DP3, (d) DP4

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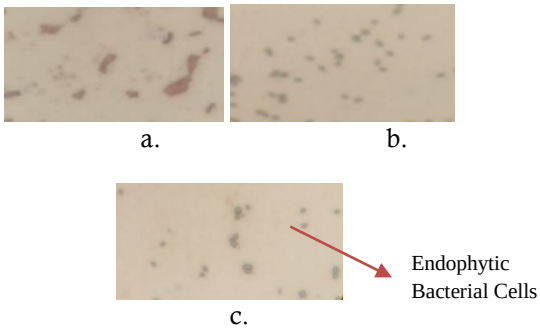

Figure 4. Morphology of Root isolates (a) AP1, (b) AP2, (c) AP3.

Antibacterial Test of Endophytic Bacteria which have potential as Antibacterials

Screening of endophytic bacteria that have potential antibacterial content showed seven isolates of endophytic bacteria that have antibacterial activity against S. aureus and Escherichia coli, namely isolates DP1, DP2, DP3, DP4, AP1 AP2, AP3

Table 2 . Diameter of Inhibition Zone of Seaweed Endophytic Microbe Isolate (*Padina* sp) against Test Bacteria E. coli and S. aureus

Bacterial Isolate Endophytes	**Inhibition Zone *Escherichia coli* (Mm)**	**Inhibition Zone *Staphylococcus aureus* (Mm)**
DP1	10,0	10,1
DP2	8,29	9,75
DP3	7,81	10,7
DP4	8,0	10,75
AP1	7,15	11,85
AP2	7,85	11,25
AP3	8,12	11,55

Note: DA = Leaf, AK = Root, BT = Bricking

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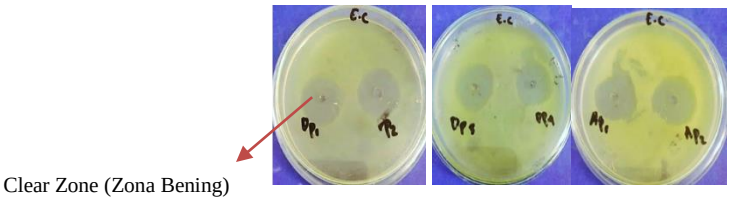

Figure 5. Diameter of the zone of inhibition of endophytic bacteria against *Escherichia coli*

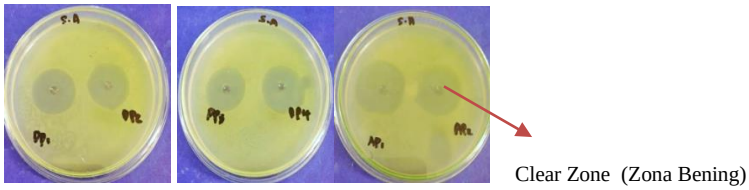

Figure 6. Diameter of the zone of inhibition of endophytic bacteria against *Staphylococcus aureus*

DISCUSSION

According to Lawalata et al., (2010) Colonies of endophytic bacteria obtained from isolation for two days where the initial stages were embedding pieces of seaweed leaves and roots in Nutrient Agar media. The isolates obtained consisted of seven isolates, namely four isolates from seaweed leaves DP1, DP2, DP3, DP4 and three isolates from seaweed roots AP1, AP2, AP3. The results of the antibacterial test found that the seven isolates of endophytic bacteria had antibacterial ability against E. coli bacteria. and S. aureus is a gram-negative bacteria. Bacteriocins produce clear zones that are bright, round and wide, so if the zones that appear are not the same then it is thought that the clear zones that appear are caused by acid, hydrogen peroxide or diacetyl activity (Lawalata et al., 2019).

The results of the antibacterial test, obtained one bacterial isolate that made the largest clear zone against E.coli bacteria compared to the other six bacterial isolates, namely Isolate DP1 this isolate made a clear zone of 8.55mm and for S. aureus microbes that made a clear zone the largest compared to the other seven isolates is isolate AP3 of 11.55 mm. The clear zone shows the potential of these bacteria to react to the secretion of bioactive compounds in the media for the benefit of survival by inhibiting the growth of other microbes around it (Syahrurahman et al., 2010; Kasi et al., 2017).

The different results in the diameter of the clear zone could be due to several factors, for example, the conditions during incubation, the process of endophytic microbial growth, and the stability of the antibacterial media. In Table 1, the isolates DP1 and AP1 are isolates that have the largest diameter of the clear zone compared to the other five isolates. The results of the antibacterial activity test results of endophytic bacteria for twenty-four hours showed that the seven isolates that had the ability as antibacterial against E. coli were isolates DP1 (10 mm), DP2 (8.29 mm), DP3 (7.81 mm), DP4 (8.0 mm), AP1 (11.85 mm), AP2 (11.25 mm) and AP3 (11.55 mm). and those that have potential on S. Aureus bacteria are DP1 (10 mm), DP2 (8.29 mm), DP3 (7.81 mm), DP4 (8.0 mm), AP1 (11.85 mm), AP2 (11.25 mm) and AP3 (11.55 mm).

The yield of 2 clear zone diameters for each endophytic bacterial isolate could be due to several factors. According to Soedarto (2015), some of the factors that influence the formation of the diameter of the clear zone are due to the process of growth of endophytic microbes and can be due to the sensitivity of pathogenic bacteria to antibacterial compounds found in endophytic microbes that affect the diameter of the resulting clear zone (Rahmi et al, 2015; Husni et al., 2014). According to Soedarto (2015); Pasappa (2022), there were different results on the diameter of the clear zone on the sensitivity of E.coli and S. aureus bacteria to antibacterials which were influenced by the structure of the gram-positive and gram-negative microbial cell walls. Gram-positive bacteria are antibacterial, because the structure on the cell walls of gram-positive microbes is simpler than the structure on the cell walls of gram-negative microbes. This difference makes it easier for antibacterial compounds to enter gram-positive microbial cells (Fithriyah, 2015); (Susanto, 2003). The presence or absence of an antibacterial activity in isolates of endophytic bacterial colonies can be caused by several factors, including the thickness of the agar media, the pH of the media, the composition of the medium, the temperature and also the incubation time (Alviana, 2016).

The seven endophytic bacterial isolates were characterized macroscopically by observing the shape, color, edges, size and elevation of the endophytic bacterial isolates (Aliah et al., 2016). The different characterization of colonies is based on table 3. DP colonies have a point shape (punctiform), the appearance of the colony (glossy) is shiny, the edges are flat (entire), the texture of the colony (viscous) is thick and has a color (colour) milky white with a surface shape (raised) whose height looks slightly visible but evenly distributed in all parts of the surface. BT colonies have the shape of a colony point (punctiform), the appearance of the colony (glossy) is shiny. Morphological or physiological characterization has its goals, namely by looking at the shape of the colony, the color of the colony, the edges and elevation of the colony (Karimela & Frans, 2020). Biochemical characterization was carried out with gelatin hydrolysis test, catalase test, citrate test, starch hydrolysis test, Indole Motility Sulfate test. The seven endophytic bacterial isolates that had been identified in terms of morphological, physiological and biochemical characterization, six endophytic bacterial isolates found six species that matched according to Jawetz & Melnick, 2015) Isolate DP1 has similarities with the genus Rhizobium leguminosarium, Isolate DP2 has similarities with the genus Corynebacterium, Isolates DP3 and AK1 have similarities with the genus Alcaligenes sp, isolate AP1 isolate DP4 has similarities with the genus Paracoccus alcaliphilus while isolate AP2 has similarities with the genus Stoptylococcus. AP3 isolate has similarities with the genus Proteus (Arlita et al., 2013).

CONCLUSION

Isolate of endophytic bacteria that has potential as an antibacterial with the largest diameter of the inhibition zone because it is suspected to have more antibacterial compounds than the other isolates, namely isolate AP3 with a diameter of (11.55) mm against S.aureus and isolate DP1 (10.0) mm against E.coli bacteria. AP3 and DP1 isolates had a larger zone suspected because their antibacterial properties were higher than the other isolates

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