Isolation and Identification of Endophytic Microbes from *Eucheuma cottonii* of North Galesong Sea, Takalar District

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Abstract. . This research aims to isolate and identify the endophytic microbes that potentially produce antibiotics from *Eucheuma cottonii*. Isolation was carried out using Pour plate method with serial dilution technique 10^{-1} to 10^{-3}. The gram stains provided excellent ways to identify the endophytic microbe. The results showed that all obtained bacteria were Gram negative and rod-shaped. There were two active endophytic microbe isolated in Agar Nutrient medium: *Aeromonas* sp. and *Klebsiella* sp.

Keywords: *Eucheuma cottonii, Endophytic Microbes, Aeromonas sp., Klebsiella sp., Seaweed*

1 Introduction

Approximately 78 percent of Indonesia's territory consists of the sea, whose beaches are rich in various types of biological sources, and their potential environment [1][2]. This situation is one of the factors that can achieve success in the fisheries sector. Marine algae cultivation is type of aquaculture in the marine sector that has the opportunity to be developed in Indonesian waters. Compared to other countries, Indonesia is a country provided many types of marine algae [3][4].

North Galesong is a sub-district in Takalar district, which has a broad sea area of nearly 15,000 hectares and became the centers of seaweed in South Sulawesi. Seaweed in Takalar is cultivated with a long line system planted in the sea area in North Galesong. Seaweed produced includes *Eucheuma cottonii*, *Gracilaria* sp., *Caulerpa racemosa, Eucheuma spinosum*, *Sargassum* sp. and *Ulva lactuca* [5][6][7].

Endophytic microbes have been isolated from *Eucheuma cottonii* tissue in previous studies and grown on media with certain compositions [8][9]. These microbes assist the absorption process of nutrients needed by plants for photosynthesis and protect their host against many of pathogens by producing mycotoxins, enzymes, and antibiotics [10][11][12][13]. The result of photosynthesis process will immediately complete their cycle through some mechanisms.

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Based on its potential, it is necessary to explore endophytic microbes isolated from *Eucheuma cottonii*, especially in North Galesong Sea. A new antibiotic can be utilized to kill drug-resistant bacteria, generally regarded as safe for treatment in human, and the price is more affordable.

2 Material and Methods

2.1 Preparation of *E. cottonii*

The sample used was fresh marine algae *E. cottonii* from Batu-Batu village, north Galesong Takalar District, South Sulawesi. Samples were taken using sterile plastic which was then put into an ice flask. *E. cottonii* is washed with seawater after being separated from the parts that are not needed then cleaned of impurities that are attached by using running water for 10 minutes and cut into small pieces. Then sterilized by soaking with 70% alcohol for 1 minute, and rinsing with distilled water. As much as 2 grams of *E. cottoni* are mashed using a dry blend, 1 gram of sample dissolved in 10 ml of sterile water and dilution until dilution of 10^{-3}.

2.2 Cultivation of Endophytic Microbes *E. cottonii*

The sample suspension from each dilution was taken 1 ml aseptically, then put in a petri dish, NA and PDA were added and homogenized. Then incubated 24 hours 37ºC (bacteria) and 28ºC (mushrooms) for 5 days. One ose of bacterial and fungal colonies was taken which showed the presence of microbes in NA and PDA mediums. A selective medium PCA was using to purified for gram-negative bacteria, TCBS in the petri dish then incubated for 1 x 24 hours at 37°C.

2.3 Isolation and Purification of Endophytic Microbes

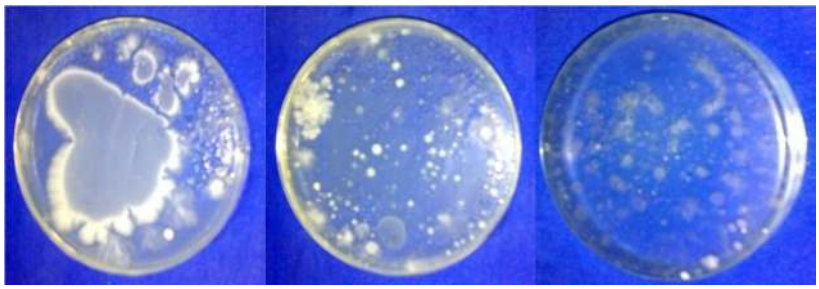
After incubation, the colonies were observed, one ose was taken and then applied to the KIA medium on the other petri dish and then incubated 24 hours. Furthermore, 1 separate colony was transferred to KIA medium and incubated for 24 hours (bacteria) and 5 days (fungi), until pure microbial colony cultures were obtained.

2.4 Gram staining

The slide was cleaned with 96% alcohol and then fixed above the spiritus lamp. Active isolates were taken aseptically and placed on a slide and then flattened. A crystal violet was applied for 1 minute (3 drops) then washed with water and dried in air. Then followed by dropping iodium for 1 minute, 96% alcohol for 30 seconds, and safranin for 45 seconds. Excess water is removed with absorbent paper. The presence of cell shape and color as seen with the microscope at certain magnification.

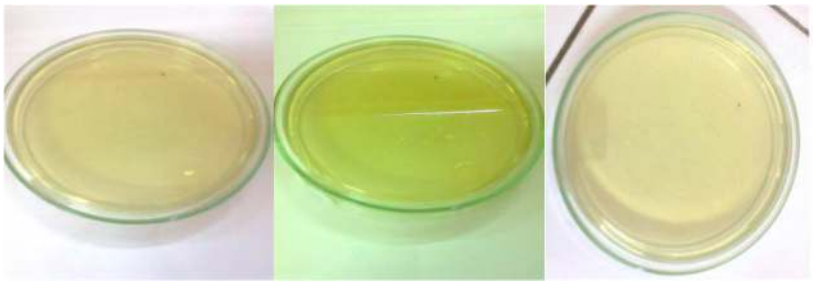
3 Results and Discussion

The results showed that two bacterial isolates showed the inhibition zones around them at 10^{-2} and 10^{-3} dilutions.



A B C

Fig 1. Isolate of endophytic bacteria from *Eucheuma cottonii* on NA
(A = 10^{-1}, B = 10^{-2}, C = 10^{-3})



A B C

Figure 2. Isolate of endophytic fungi from *Eucheuma cottonii* on PDA
(A = 10^{-1}, B = 10^{-2}, C = 10^{-3})

The isolation method used is the pouring with serial dilution technique. This is intended to reduce the number of microbes, to obtain a good spread of colonies and not to accumulate. Both NA and PDA medium containing nutrients for growth of endophytic microbes. In the NA, 2 isolates were obtained, A1 and A2, whereas in the PDA does not indicate isolates as seen in Figure 2. The isolates obtained were then purified by streaking using the quadrant method on selective medium, BA medium for gram-positive bacteria, MCA for gram-negative bacteria, TCBS for *Vibrio* sp. There were no isolates grew in BA medium, no gram-positive bacteria survive in these media. Pure isolates were streaked onto KIA to facilitate the identification and also as a stock.

Gram staining was performed to classify as gram-positive or gram-negative bacteria. The fixation refers to the process of attaching cells to a slide [14], kill microbes rapidly without causing changes in shape and structure, change the binding capacity of dyes, make the cells of microbes more stable, release granular proteins into reactive groups, and prevent cell rupture caused by their enzymes [15][16].

Gram staining used dye A, dye B, dye C, and dye D. The dyes used are an organic mixture containing a chromophore and autochrome group that helps in benzene rings. Gram-positive bacteria remain purple refers to the thick layer retain the color of violet-iodine crystals even though the solution is bleached. While gram-negative bacteria thus appear red or pinkish. The thin layer of peptidoglycan is unable to retain the crystal violet-iodine complex and dissolves when giving a bleach solution. Gram staining results showed that all isolates were gram-

negative and rod-shaped bacteria. Based on morphological observations, these bacteria are *Aeromonas* sp. and *Klebsiella* sp.

4 Conclusion

Isolation of endophytic microbes from marine algae *Eucheuma cottonii* found in North Galesong Sea produce rod-shaped and gram-negative bacteria. These bacteria are *Aeromonas* sp. and *Klebsiella* sp.

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